

BD Biopharmaceutical Production

BIONUTRIENT TECHNICAL MANUAL

SECOND EDITION



BD

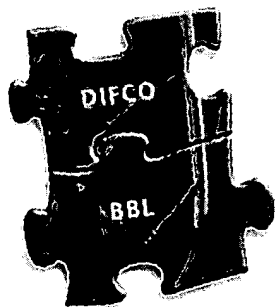
Helping all people
live healthy lives

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INTRODUCTION



The BD Difco™ and BBL™ Bionutrient Technical Manual has been provided to assist you in your selection of BD products for use in cell culture and microbial fermentation production, from Research and Development to the final finished product.

It is our commitment to innovation and product consistency as well as our broad access to the raw material market that makes BD (Becton, Dickinson and Company) such a strong global supplier and partner. In over 142 countries worldwide, BD offers a full line of bionutrients and media, for the biotechnology, pharmaceutical, animal and human vaccine, and bioremediation markets. BD offers products for both cell culture and microbial fermentation production, as well as applications for industrial research, QA/QC and environmental monitoring.

Capability

Our commitment to the cell culture and fermentation media market is exemplified in our wide range of capabilities:

- **Largest media manufacturing plant in the world**—In 1999, as part of the Difco Laboratories acquisition, BD opened a manufacturing plant in Sparks, Maryland, which is our Center of Manufacturing Excellence. With over 101,000 sq. ft. of production capacity, the center is a state-of-the-art modern facility that manufactures dehydrated culture media, prepared culture media, bionutrient ingredients, stains, and other products for microbiological and cell culture use worldwide.
- **Full line of meat peptones**—Building on the reputation of the Difco meat peptones, BD continues to operate the previous Difco manufacturing facility in Detroit as a source for the high quality Difco™ and Bacto™ brand products. This commitment to tradition carries on the quality and performance established under the Difco name, long recognized throughout the industry for superior quality. BD continues investing in Research and Development for peptone products, which continually expands our understanding of their application in cell culture and microbial fermentation.
- **Expanding line of animal-free products**—As early as 1998, BD started offering animal-free products to the fermentation industry, introducing its Select APS™ (Alternative Protein Source) Super Broth, Select APS LB Broth, and Select Soytone. BD continues to leverage its expertise in creating high performing animal-free products to meet evolving customer needs in the cell culture and fermentation industry with the introduction of Difco™ Springer™ DS 100 Soy Peptone UF.

Today, BD Offers the following Animal-Free Products

- Phytone™ Peptone
- Phytone™ Peptone UF
- DS 100 Soy Peptone UF
- Select Soytone
- Bacto™ TC Yeastolate
- TC Yeastolate UF
- Bacto™ Yeast Extract
- Yeast Extract
- Yeast Extract UF
- Yeast Extract LD
- Select APS™ LB Broth
- Select APS™ Super Broth
- Bacto™ Malt Extract
- M9 Minimal Salts, 5x
- Yeast Nitrogen Base
- Yeast Nitrogen Base w/o Amino Acids
- Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

- **Custom Media Program**—BD has available a custom media program to meet individual customer requirements. The program offers three levels of customization, from special packaging and QC testing requirements to full formula optimization services. It is our goal to service our customers with the highest level of technical support and manufacturing flexibility.
- **Media Optimization Program**—At the highest level of technical interaction, the Custom Media Program allows customers to outsource media optimization to BD, thus allowing them to focus resources in other critical areas. By accessing BD expertise in media development, customers can take advantage of our experience in yield enhancement and media formulation scale-up.
- **Dedicated animal-free equipment and environment**—With the rising concerns over bovine spongiform encephalopathies and transmissible spongiform encephalopathies (BSE/TSE), BD has met the challenge by dedicating process equipment to the production of animal-free products. Specific orders can be produced in this animal-free environment.
- **Heightened Regulatory Compliance**—BD plants are ISO 9001 and 13485 Certified, and regularly inspected by the FDA to conform with our cGMP manufacturing practices. We also offer the biopharmaceutical industry comprehensive programs in documenting raw material origin, manufacturing change control and Drug Master Files (DMF) for key products.

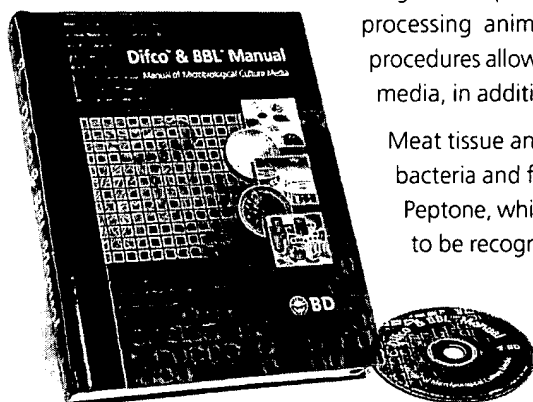
History

How did BD build this unmatched foundation to support the Biopharmaceutical industry? In 1955, BD acquired the Baltimore Biological Laboratory (BBL) of Baltimore, Maryland, and used its expertise to continually advance the clinical market with prepared media and diagnostic tools. In 1997, BD acquired Difco Laboratories of Detroit, Michigan. Since that acquisition, BD has merged Difco Laboratories and BBL Microbiology Systems into one division that provides customers with media, peptones, hydrolysates and extracts. Today, BD is one of the largest microbiology companies in the world, offering a broad range of microbiology and cell culture products worldwide.

Difco Contributions to the BD Product Line

Beginning in 1895, Difco Laboratories produced high quality enzymes, dehydrated tissues and glandular products to aid in the digestion process. The knowledge gained from processing animal tissues, purifying enzymes and performing dehydration procedures allowed a smooth transition to the preparation of dehydrated culture media, in addition to its peptones.

Meat tissue and other protein digests were developed to stimulate growth of bacteria and fungi. Extensive research led to the development of Difco Bacto Peptone, which was introduced in 1914. Since then, Bacto Peptone continues to be recognized as the 'premium quality' standard for all other peptones.



Building on this knowledge base, Difco continued to develop more peptones to add to the Bacto line of products. Bacto Proteose Peptone, Bacto Proteose Peptone No. 2, and Bacto Proteose Peptone No. 3 were created from the accumulated information that no single peptone was the most suitable nitrogen source for growing fastidious bacteria and supplementing cell culture. Today, many cell culture procedures, in addition to microbial cultures, call for the addition of a peptone to enhance yield.

Combined Strengths Build the Largest Breadth of Line

Today, having consolidated products from the Difco™ and BBL™ lines, BD not only offers peptones/hydrolysates manufactured from meat, animal tissue, collagen, gelatin and casein, but also products from animal-free materials. BD Difco brand yeast extracts are produced from primary grown (baker's) yeast and provide lot-to-lot consistency that outperforms brewer's yeast, as well as competitive products. BD is continuously expanding its peptone line of products by adding additional animal-free and plant origin peptones.

Along with combining the two premier manufacturers in the industry, BD has implemented a new branding strategy for peptones. While the Difco brand is the leading BD brand for production peptones, the BBL brand will remain on certain key BBL formulations. The BBL brand has become the leading BD brand for all prepared liquid media products.

In addition to the main Difco and BBL brands, familiar brands such as Bacto, BiTek™, and Select will also continue. The Difco Bacto brand has been retained for the traditional Bacto labeled products, while maintaining the integrity of the original premium Bacto formulas. The Difco BiTek brand has been continued for the production grade products where a premium product is not required. The Select brand remains for certain original animal-free BBL formulas.

The consolidated BD BBL and BD Difco product lines provide quality products with lot-to-lot consistency, backed up by BD service, support, and custom programs to address individual requirements. All this, combined with our proactive responses to BSE/TSE concerns makes BD stand out as the best choice for fermentation and cell culture ingredients.

Product Quality

In our effort to reduce BSE/TSE issues, BD sources raw materials for all products from known BSE-free countries—U.S.A., New Zealand, and Australia. All raw materials are tested upon receipt to assure that they meet BD incoming specifications. Then final products are tested prior to release to assure quality and consistency. After final release, the products are packaged and retention samples are held for stability studies and any additional testing required at a later date.

Certificates of Analysis and Certificates of Origin for each product contain all the information required for complete traceability of all raw materials included in each product. For your convenience, these certificates are available from the BD web site 24 hours a day, 7 days a week, at www.bdregdocs.com.

Service

BD maintains inventory in our BD Distribution Centers in Sparks, Maryland, and Temse, Belgium. With multiple manufacturing locations, BD is prepared to provide products and support to handle any need or situation.

For global organizations, BD offers a formal global interacting capability to manage a single contract for multiple locations on a global basis. Please contact your local BD representative for details of the Global Key Account Program, or our web site at www.bd.com/ds.

At BD, we are continually adding new products. Please contact us if you have a need for a product that you do not find in this Bionutrient Technical Manual. Our Technical Services Group and Research and Development team are available to work with you and support your media requirements.

Using This Manual

As presented in this manual, BD offers a wide range of products in the following categories:

- Animal-free Peptones, Yeast Extracts and Media
- Meat Peptones and Media
- Casein Peptones

The manual provides insight into both cell culture and fermentation applications. Each product description contains data on physical characteristics, chemical analysis and amino acid distribution. A complete listing of regulatory services is provided and an alphabetical listing of products appears in the back of the manual.

Thank you for your past and continued business.

HYDROLYSIS TO HYDROLYSATE

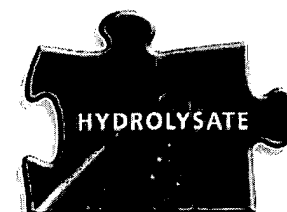
Proteins are molecules essential to the structure and function of all living organisms. They are made up of chains of any number of amino acids linked by peptide bonds and folded in a variety of complex structures. Proteins may be broken down into amino acids and peptides by hydrolysis using strong acids or proteolytic enzymes such as pepsin, papain, or pancreatin, which contains trypsin and chymotrypsin. These protein hydrolysates are called peptones.

The starting materials for peptones vary from animal to vegetable. Protein sources include meat, casein (milk protein), gelatin, soybean, yeast and grains. Enzyme sources include animal organs (pancreatin and pepsin), papaya (papain), fig (ficin), pineapple (bromelain) and microbes.¹

Acid hydrolysis is a harsh process, usually carried out at high temperature, which attacks all peptide bonds in the protein substrate, destroying some of the individual amino acids liberated. Tryptophan is usually totally lost in an acid hydrolysis. Cystine, serine and threonine are partially broken down and asparagine and glutamine are converted to their acidic forms. Vitamins are mostly destroyed by acid hydrolysis.

Proteolytic enzymes hydrolyze proteins more gently than acids, do not require the high temperature used for acid hydrolysis, and usually are specific to the peptide bonds they will break. The resulting material from a proteolytic digestion is a mixture of amino acids and polypeptides of varying lengths. The enzyme pepsin will cut an amino acid chain where there is a phenylalanine or leucine bond. Papain will cut the chain adjacent to arginine, lysine and phenylalanine, and pancreatin shows activity at arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine bonds.²

Microbial proteases, proteolytic enzymes secreted by microorganisms, are becoming more widely used in peptone production. Proteases from bacterial, algal, fungal and yeast sources cover a wide variety of enzyme activities, can be produced in large scale, and usually require only simple purification.³



Products by Category

Meat Peptones and Media:

Beef Extract, Powder	Bacto Proteose Peptone
Bacto™ Beef Extract, Desiccated	BiTek Proteose Peptone
Bacto Brain Heart Infusion	Bacto Proteose Peptone No.2
Bacto Brain Heart Infusion, Porcine	Bacto Proteose Peptone No.3
Gelysate™ Peptone	BiTek Proteose Peptone No.3
Bacto Neopeptone Peptone	Bacto Proteose Peptone No.4
Bacto Peptone	Thiotone™ E Peptone
BiTek™ Peptone	Bacto Tryptose Peptone
Polypeptone™ Peptone	

Casein Peptones:

Acidicase™ Peptone	Trypticase™ Peptone
Bacto Casamino Acids	Bacto Tryptone Peptone
Bacto Casamino Acids, Technical	BiTek Tryptone Peptone
Bacto Casitone Peptone	Biosate™ Peptone
Bacto TC Lactalbumin Hydrolysate	

Soy Peptones:

Phytone™ Peptone	Select Soytone
Phytone Peptone UF (Ultra Filtered)	Bacto Soytone
DS100 Soy Peptone UF	

Yeast Extracts:

Bacto TC Yeastolate	Yeast Extract
TC Yeastolate UF	Yeast Extract UF
Bacto Yeast Extract	Yeast Extract LD (Low Dusting)

Alternative Protein Source (APS)/Animal-Free Products:

DS100 Soy Peptone UF	Yeast Extract
Phytone Peptone	Yeast Extract UF
Phytone Peptone UF	Yeast Extract LD
Bacto TC Yeastolate	Select APS™ LB Broth
TC Yeastolate UF	Select APS Super Broth
Bacto Yeast Extract	Bacto Malt Extract

Chemically Defined Products:

M9 Minimal Salts, 5x	Yeast Nitrogen Base
Yeast Nitrogen Base w/o Amino Acids	Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

The raw materials and manufacturing conditions for protein hydrolysis are controlled to produce consistent peptone products. Ingredients used for peptone manufacture, including the protein, agent of hydrolysis, and any buffering agents used, are selected based on specific purity and quality standards. The conditions of the hydrolysis, such as the amount of enzyme used, the time for digestion, and the pH and temperature at which hydrolysis is conducted, determine the degree of hydrolysis and the quality of the hydrolysate. Therefore, these conditions must be carefully controlled throughout the manufacturing process. Purification, concentration and drying steps are carefully regulated due to their bearing on the characteristics of a peptone. Finally, each batch of protein hydrolysate is tested for an array of physical, chemical, analytical and growth support tests to ensure product quality and lot-to-lot consistency.

References

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Norris and Ribbons (ed.), *Methods in microbiology*, vol. 3A. Academic Press, New York.
2. Dixon and Webb. 1979. *Enzymes*, 3rd ed. Longman Group Limited, London.
3. Cowan. 1991. Industrial enzymes. *In* Moses and Cape (ed.), *Biotechnology, the science and the business*. Harwood Academic Publishers GmbH, Chur, Switzerland.

CELL CULTURE APPLICATIONS

Why Peptones in Cell Culture

In the biopharmaceutical industry, concerns over using animal-derived components have prompted investigation into new forms of supplementation. Traditionally, cell culture media have been supplemented by the addition of serum or serum-derived components. While this helped to complete the diverse nutritional and growth requirements of the cells, the high product cost and variability between lots were difficult obstacles to overcome. Concerns over infectious agents such as viruses, mycoplasma, and prion diseases like BSE/TSE demonstrated the need for an alternative form of supplementation such as animal-free peptones.¹ For over 30 years peptones have been successfully used to replace serum (Figure 1) in various cell culture applications.²



While a completely defined cell culture medium is ideal, the cost and time associated with optimization could be prohibitive to a timely product launch. Peptones and hydrolysates provide a good supplementation alternative to completely defined media by decreasing development time while showing increased yield. The wide variety of animal-free peptones available provides many different supplementation options that will yield optimal results within a more acceptable time frame.

Peptone Selection Criteria

Since every cell line is different it is necessary to test an assortment of peptones at a variety of concentrations to optimize their performance. Multiple peptones should be included in the initial screen, even if some peptones were derived from the same starting material. Figure 2 shows the improvement in protein production for a CHO line when DS100 was substituted for Select Soytone. Figure 3 shows the differences achieved in antibody yields depending upon the peptone and concentration used. The proliferation data in figures 4 and 5 demonstrate the advantage of generating titration curves

Figure 1

Product Name	Endotoxin EU/gram	Osmolality (μOsm)*	Hypoxanthine (μg/g)*	Thymidine (μg/g)*
DS100 Soy Peptone UF	<300	45	14	<10
Phytone™ Peptone	300	51	<2	<10
Phytone™ Peptone UF	<300	52	<2	<10
Proteose Peptone No. 3, Bacto™	20	53	233	74
Select Soytone	2200	48	18	<10
TC Lactalbumin Hydrolysate	100	48	7	9
TC Yeastolate UF	<500	64	32	<10
TC Yeastolate, Bacto™	900	59	31	<10
Tryptone, Bacto™	100	45	17	16
Yeast Extract, Bacto™	100	60	24	<10
Yeast Extract, UF	<300	61	39	<10

* Values derived from an average of three lots

Figure 2

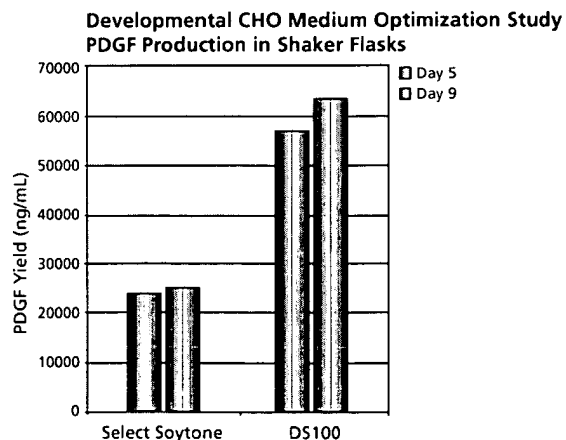
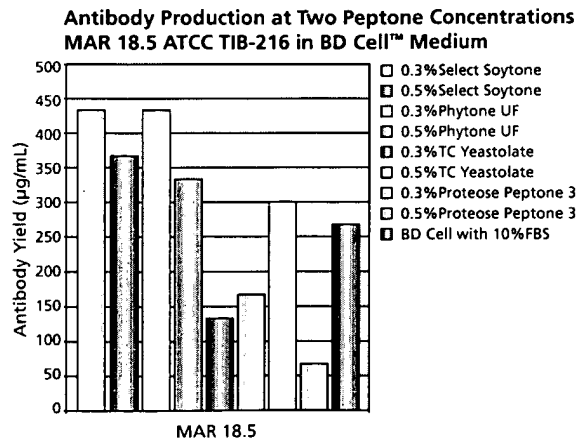


Figure 3



for each peptone that would be used with a cell line in a particular base medium. This data ensures that the peptone is being supplemented into the base medium at a concentration that leads to optimal cell performance.

The benefits achieved when peptones are used will be enhanced if they are used with an optimized base medium. Figure 6 shows the differences in antibody yield when two different peptones are used in either IMDM or BD Cell™ medium, a specialized hybridoma medium for high-level antibody yield. Previous work has shown that cells will not perform in IMDM without the proper supplementation. The level of antibody produced in IMDM with peptone supplementation was similar to the level achieved in BD Cell medium without peptone. The level in BD Cell was increased almost five-fold when peptones were added to the BD Cell medium.

Various forms of peptone supplementation such as peptone blending and feed strategies should also be considered during peptone selection. Figures 7 and 8 show blends

Figure 4

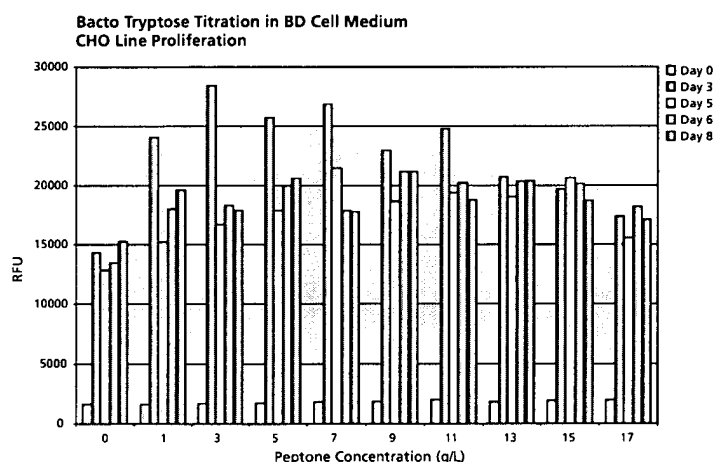
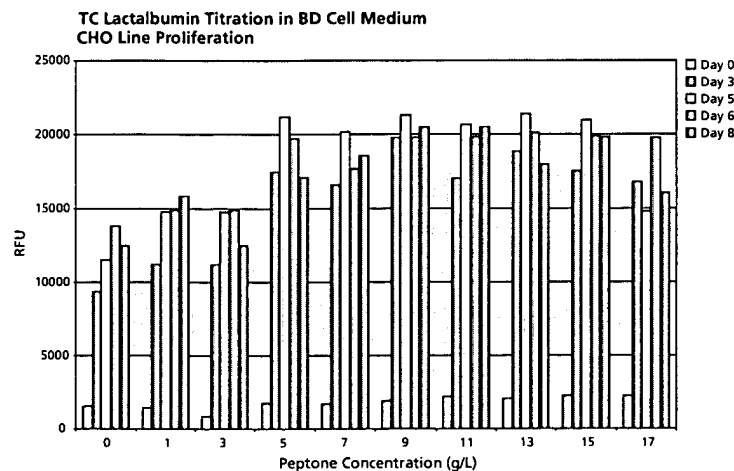


Figure 5



of Select Soytone and TC Yeastolate UF where the total quantity of peptone added to either BD Cell Medium or IMDM was 3 g/L. In IMDM a significant increase in antibody yield was achieved when Select Soytone was blended with a small amount of TC Yeastolate UF compared to the soy or yeast peptone used alone. However, blending had an inhibitory effect when it was used in a more optimized base medium like BD Cell Medium. The right combination of peptones, as well as a consideration of feed strategy, is essential in order to optimize every portion of the process. Since lot-to-lot consistency is always a concern when using peptones, evaluations of multiple lots and a clear understanding of the product specifications is critical.

Purification requirements are greatly reduced when peptones are used as a replacement for serum or any of its derived proteins. The gel in Figure 9 shows the differences in unpurified supernatants of hybridoma cells grown

Figure 6

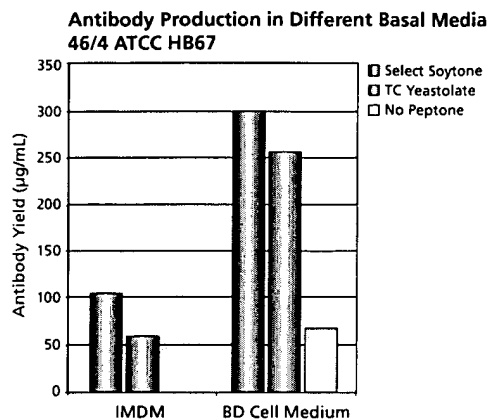


Figure 7

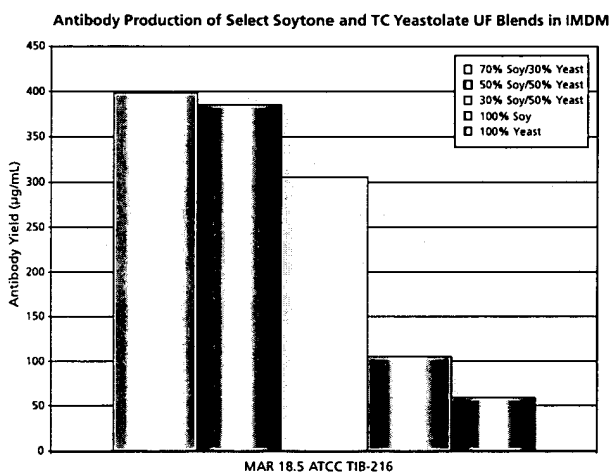


Figure 8

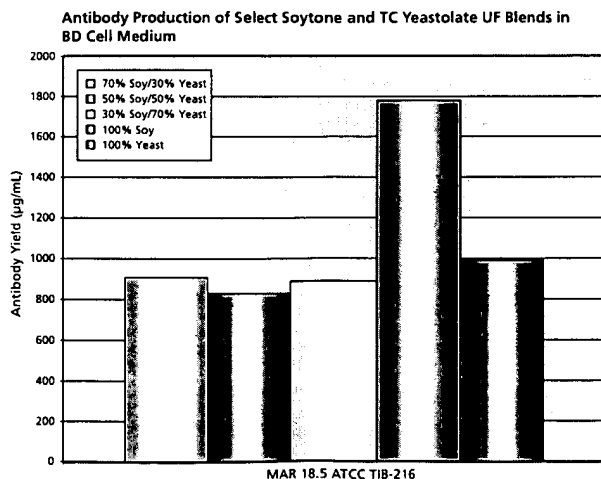
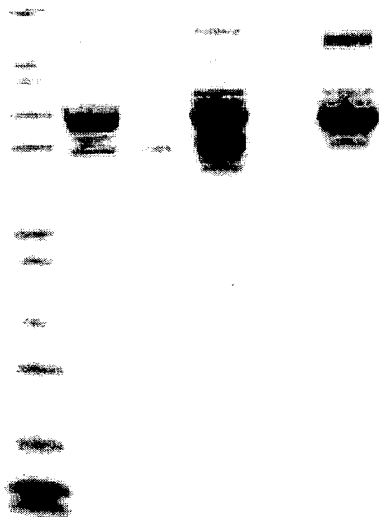


Figure 9

Lane 1 Lane 2 Lane 3 Lane 4 Lane 5 Lane 6
2.5-200 kDa



in variously supplemented media. Lane 4 shows the large amount of contaminating protein that is present when 10% serum is used as a supplement. While the samples supplemented with serum-derived components in Lanes 2 and 6 are cleaner, a great deal of purification is still required. The medium where a peptone was used as a replacement for serum (Lane 3) is just as clean as the protein-free medium used as a control (Lane 5).

BD Brand Peptones Meet Your Needs

Media optimization using peptones provides a way to increase cell proliferation and production by creating an ideal environment specific to the cell within a more acceptable development time. Performing a wide screen of peptones used individually or in combination is essential in order to determine the optimal conditions for a particular cell line. Typically, performing such a broad and inclusive peptone screen is labor intensive and time consuming. In order to decrease development time BD has automated these methods to ensure that all possible variables are evaluated, resulting in the selection of the best supplementation scheme.

Regardless of the application need, BD peptones are available to help the user reach their research or production goals. While the concentration used should be optimized for each system, a starting concentration of 1-10g/L is recommended.

References

1. Heidemann, Zhang, Qi, Rule, Rozales, Park, Chuppa, Ray, Michaels, Konstantinov and Naveh. 2000. The use of peptones as medium additives for the production of a recombinant therapeutic protein in high density perfusion cultures of mammalian cells. *Cytotechnology* 32:157-167.
2. Taylor, Dworkin, Pumper and Evans. 1972. Biological efficacy of several commercially available peptones for mammalian cells in culture. *Exptl Cell Res* 74:275-279.

Peptones for Cell Cultures

Product	Substrate	Applications
Phytone™ Peptone	Soy	Excellent for growth promotion and protein production, as well as a good, animal-free alternative to serum. (Page 45)
Phytone™ Peptone UF	Soy	An ultrafiltered version of Phytone with a low endotoxin specification. (Page 45)
Proteose Peptone No. 3	Porcine	Excellent for growth promotion and protein production, as well as a good alternative to serum. (Page 23)
Select Soytone	Soy	Excellent for growth promotion and protein production. (Page 45)
DS100 Soy Peptone UF	Soy	An ultrafiltered soy hydrolysate suitable for serum replacement in cell culture applications. (Page 45)
Tryptose Peptone	Meat	Excellent serum-free supplement for human diploid fibroblasts. (Page 28)
TC Lactalbumin	Milk	Excellent for amino acid supplementation. (Page 11)
TC Yeastolate	Yeast	Good for growth promotion, especially with insect cells. (Page 48)
TC Yeastolate UF	Yeast	An ultrafiltered version of TC Yeastolate with a low endotoxin specification. (Page 48)
Bacto™ Yeast Extract	Yeast	Good for growth promotion, especially with insect cells. (Page 49)
Yeast Extract UF	Yeast	An ultrafiltered Yeast Extract with a low endotoxin specification. (Page 49)

For more animal-free product information see page 42.

BACTO™ TC LACTALBUMIN HYDROLYSATE

Product Description

Bacto™ TC Lactalbumin Hydrolysate is the enzymatically hydrolyzed protein portion of milk whey, which is recognized as a complete protein source. This product is a mixture of peptides, amino acids and carbohydrates, both simple and complex, used for preparing bacterial, insect and mammalian cell culture media.

Applications

Bacto TC Lactalbumin Hydrolysate is intended as a nutritional supplement for bacterial, insect and mammalian cell culture. For years, TC Lactalbumin Hydrolysate has been used as a nutritional source for lactobacilli. It is also useful for indole testing because of its high tryptophan content. TC Lactalbumin is frequently used in mammalian cell culture media as an amino acid supplement.¹

Physical Characteristics

Bacto TC Lactalbumin Hydrolysate is a homogeneous, free-flowing, dehydrated powder, buff to tan in color.

For Typical Analyses, see page 40.

Availability

Bacto™ TC Lactalbumin Hydrolysate 259962, 500 g

Bacto™ TC Lactalbumin Hydrolysate 259961, 10 kg

Reference

1. Artemenko, Ivanova, Nenashev, Kuznetsova and Ochkina. 1985. Use of experimental analytical method for equilibrating nutrient broths for *Clostridium perfringens* type A growth and toxin production. Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii. 11:37-41.

FERMENTATION APPLICATIONS

Defined vs. Complex Media

Fermentation media formulations are of two types: defined and complex. Defined media are made by the addition of chemically-defined ingredients to water for injection (WFI) or distilled water. Complex media are made with peptone

digests or extracts of plant or animal origin (see "Hydrolysis to Hydrolysate").¹ The advantages of chemically-defined media are greater repro-

ducibility, cleaner downstream processing steps and simplicity in the analysis of the end product. The disadvantages are lower yields and greater expense, especially if the list of media components include growth factors and vitamins.² The advantages of complex media are that they are relatively inexpensive, they support a wide variety of growth from a large group of microorganisms, they promote growth of the more fastidious organisms that will not grow on a chemically-defined medium, they stimulate toxin production and they routinely produce higher yields than many defined media. The disadvantages of complex media are that the downstream processing may be more difficult and reproducibility can sometimes be compromised.

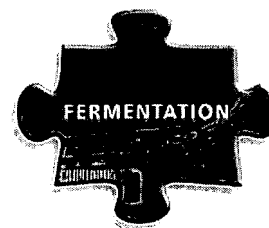


Figure 1

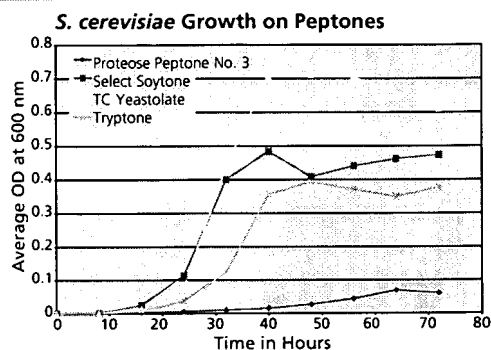


Figure 2

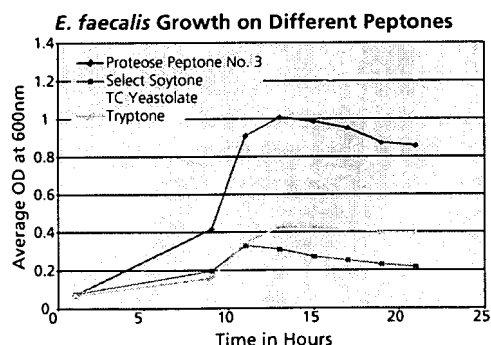
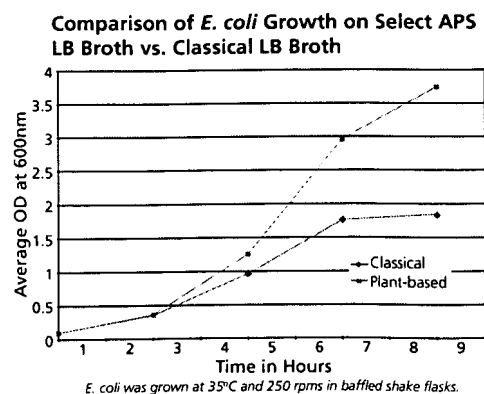


Figure 3



Selecting a Peptone

Successful media development is a multifaceted process. In order to comprehensively cover all the variables with the least time and effort it is usual to employ statistical methods.³ When developing a new formulation, care should be taken in choosing the peptones for the new formulation. Individual experimentation with a variety of peptones is suggested to select the optimum peptone or combination of peptones. Figures 1 and 2 demonstrate such a preliminary screen for multiple peptones and two different organisms. Each peptone was prepared as 1% solution with 0.4% glucose and buffering salts. Growth testing was performed using the Labsystems Bioscreen C Kinetic Optical Density Reader. Review of the growth support curves for Proteose Peptone No. 3 illustrates poor growth support (Figure 1), while Figure 2 shows good growth support. Based on these results, one would likely create a formulation consisting of TC Yeastolate and Proteose Peptone No. 3 to support the growth of *Enterococcus faecalis*.

Moving From Animal to Animal-Free

With the continuing emergence of new confirmed cases of BSE/TSE, a prime directive for the development of new fermentation products has been to either source raw materials from a country free from BSEs or reformulate the media using animal-free components.⁴

BD began reformulating animal component media formulations to animal-free formulations in 1997. In 1998, we introduced our line of Select APS™ (alternative protein source) products. In the case of the LB Broth reformulation, the performance of the test organism, *E. coli* DH5α was enhanced (Figure 3). The experiment was conducted in a shake incubator set at 250 rpm and 35°C.

Another example of enhanced performance is demonstrated in Figure 4. *E. faecalis* was grown side-by-side in New Brunswick BioFlo 3 fermentors. In this case growth enhancement, as measured by the mass or OD reading, doubled when the medium formulation was changed to all animal-free components.

Figure 5 shows growth curves for two of the five different soy peptones available from BD. It also demonstrates the differing responses an organism may have to different peptones made with the same starting materials. For an *E. coli* with a plasmid, the Select Soytone provides better growth support than Trypticase™ Peptone. In this experiment the peptones were in 2% solutions with some buffering salts. The purpose of the experiment was to observe what type of growth support the individual peptones contributed to a multicomponent medium.

Figure 6 demonstrates the rigorous quality control testing these media undergo. Three lots of Select APS™ Super Broth were growth tested using an *E. coli* strain containing a plasmid. The three growth curves are nearly identical in their growth support. Product consistency is demonstrated through the quality control testing of the final product.

References

1. Demain and Solomon. 1986. Manual of industrial microbiology and biotechnology, p.108-109. American Society for Microbiology. Washington, D.C.
2. Cote. 1999. Media composition, microbial, laboratory scale. In Flicker and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York.
3. Haaland, 1989. Experimental Design in Biotechnology, Marcel Dekker, New York.
4. Baron, Safar, Groth, DeArmond and Prusiner. 2001. Prions. In Block (ed.), Disinfection, sterilization, and preservation, 5th ed. Lippincott Williams & Wilkins. Baltimore.

Figure 4

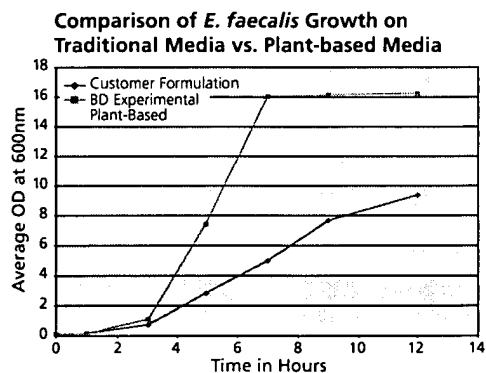


Figure 5

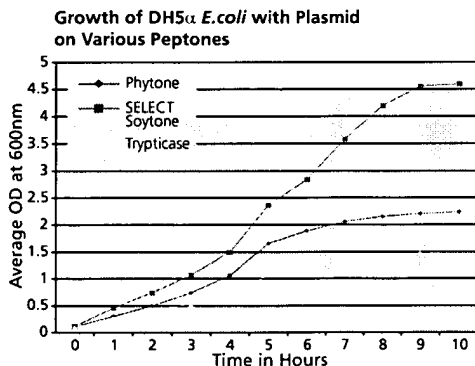
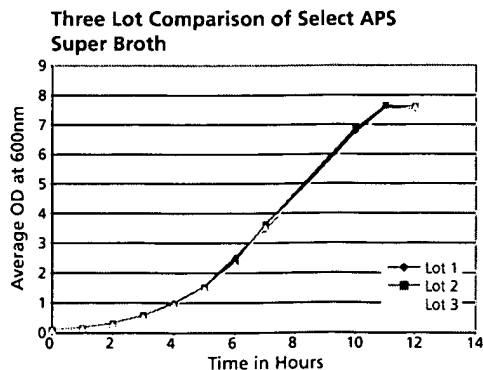
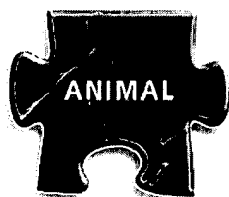


Figure 6



MEAT PEPTONES AND MEDIA



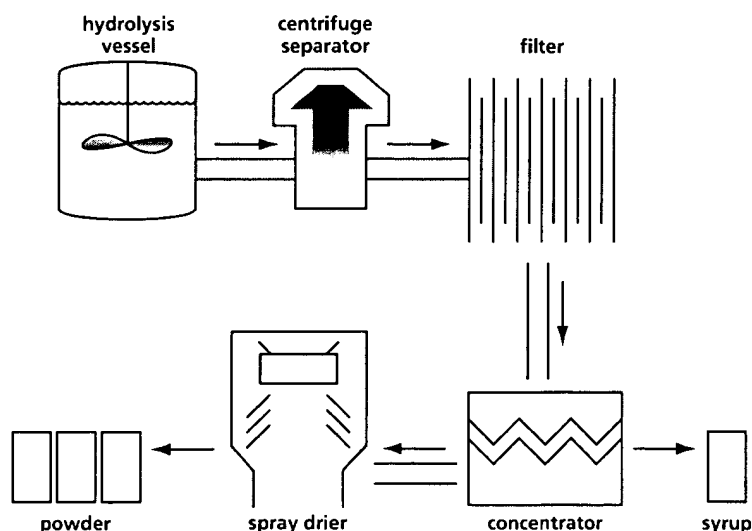
Meat peptones are proteins from animal sources that have been hydrolyzed, or broken down into amino acids and peptides, to provide nitrogen for microorganisms. Meat peptones can be tailored to specific nutritive needs of microorganisms by controlling the quality and origin of the protein, the quality and source of the enzyme used to digest the protein, and the methods used for hydrolysis, concentration and drying the peptone.

Sources of animal protein include meat from muscle tissue or offal (waste parts, entrails) and gelatin. Muscular tissue and offal are utilized fresh, frozen or dried. Gelatin is extracted by boiling collagen, the fibrous protein found in connective tissue, bone and cartilage.

A variety of proteolytic enzymes, or proteases, may be used to accomplish enzymatic hydrolysis of animal protein. Pepsin and trypsin are widely used for animal peptone manufacture. Pepsin is isolated from porcine or other animal stomach. Trypsin, along with chymotrypsin, carboxypeptidase A, carboxypeptidase B, and elastase, are enzymes isolated from animal pancreas.

Peptone manufacture includes the following steps: hydrolysis/digestion, centrifugation, filtration, concentration and drying. Animal tissues are chopped in order to prepare for digestion, and demineralized water is added to the starting constituent(s) to form a thick suspension of protein material. The material is placed in large-capacity digestion vessels, which are stirred continuously. Base or acid is added to bring the pH of the protein suspension to the optimum for the specific enzyme being used. For example, pepsin is most effective at pH 2.0 and trypsin shows maximum activity at pH 8.5.¹ The enzyme is added when the pH and temperature are optimal. The amount of enzyme necessary, time for digestion, and control of pH and temperature are dependent on the degree of hydrolysis intended.

Diagram of Manufacturing Process



Once protein digestion is complete, the suspension may be heated to inactivate the enzymes. The protein/enzyme slurry is then centrifuged and/or filtered to remove the insoluble materials and to clarify and concentrate the material. The peptone solution may be vacuum-evaporated to rapidly concentrate the peptone. The peptone syrup, which contains approximately 67% solids, may undergo further processing for pH adjustment or filtration. The final drying step of the process further concentrates the protein by spray-drying or by pan-drying in vacuum ovens, which readies the material for packaging.

Reference

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. In Norris and Ribbons (ed.), *Methods in microbiology*, vol. 3A. Academic Press, New York.

BEEF EXTRACT POWDER

BACTO™ BEEF EXTRACT, DESICCATED

Product Description

Beef Extract is derived from infusion of beef and provides an undefined source of nutrients. Beef Extract is not exposed to the harsh treatment used for protein hydrolysis, so it can provide some of the nutrients lost during peptone manufacture.¹ Beef Extract is a mixture of peptides and amino acids, nucleotide fractions, organic acids, minerals and some vitamins. "Its function can therefore be described as complementing the nutritive properties of peptone by contributing minerals, phosphates, energy sources and those essential factors missing from peptone."² Beef Extract Powder is a meat extract dried to powder form. Bacto™ Beef Extract, Desiccated, is the dried form of Beef Extract paste.

Applications

Beef Extract is intended to replace aqueous infusion of meat in microbiological culture media. Beef Extract is frequently used at a concentration of 0.3 to 1.0% in culture media, although concentrations may vary depending on the nutritional requirements for the medium formulation.

Beef Extract was used in media for early studies of non-sporulating anaerobes of the intestinal tract and as a stock broth in the study of nutritional needs of streptococci. Prokofeva et al.³ used Beef Extract for growing thermoacidophilic organisms newly isolated from hot springs in Kamchatka, Russia. Kataoka and Tokiwa⁴ used Beef Extract as a nitrogen source in studies of mannose production by *Clostridium tertium* strains isolated from soil and methanogenic sludge. In addition, Beef Extract is a nutritive ingredient in many classical culture media, including Antibiotic Assay media described in *The United States Pharmacopeia*,⁵ and several media recommended for standard methods applications.⁶⁻⁸

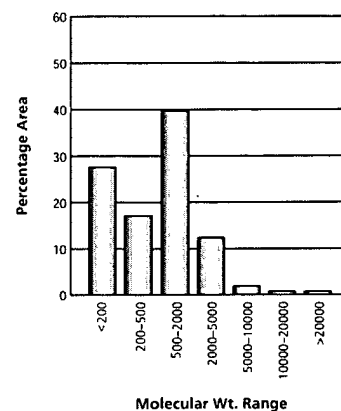
Physical Characteristics

Beef Extract Powder is a light to medium, cream to tan, free-flowing, homogeneous powder.

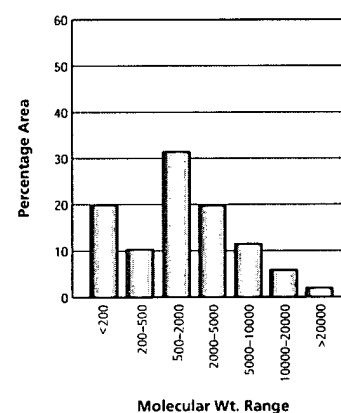
Bacto Beef Extract, Desiccated is a medium to dark brown, crystalline powder.

Molecular Weights

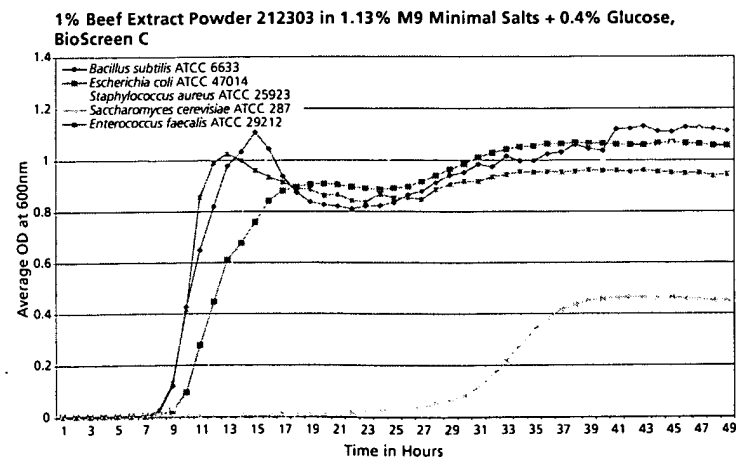
Beef Extract Powder



Bacto Beef Extract, Desiccated



Growth Curve



Availability

Beef Extract Powder 212303, 500 g

Bacto™ Beef Extract, Desiccated 211520, 500 g

References

1. Cote. 1999. Media composition, microbial, laboratory scale. In Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York.
2. Bridson and Brecker. 1970. Design and formulation of microbial culture media. In Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.
3. Prokofeva, Miroshnichenko, Kostrikina, Chernyh, Kuznetsov, Tourova and Bonch-Osmolovskaya. 2000. *Acidilobus aceticus* gen. nov., sp. nov., a novel anaerobic thermoacidophilic archaeon from continental hot vents in Kamchatka. Int. J. Syst. Evol. Microbiol. 50: Pt 6:2001-2008.
4. Kataoka and Tokiwa. 1998. Isolation and characterization of an active mannanase-producing anaerobic bacterium, *Clostridium tertium* KT-5A, from lotus soil. J. Appl. Microbiol. 84:357-367.
5. United States Pharmacopeial Convention. 2004. The United States pharmacopeia 27/The national formulary 22—2004. United States Pharmacopeial Convention, Inc., Rockville, Md.
6. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, DC.
7. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC.

BACTO™ BRAIN HEART INFUSION

BACTO™ BRAIN HEART INFUSION, PORCINE

Product Description

Bacto™ Brain Heart Infusion (BHI) is a microbiological culture medium used for cultivating fastidious microorganisms, including streptococci, pneumococci and meningococci. In 1919, Rosenow¹ devised an excellent medium for culturing streptococci by supplementing dextrose broth with brain tissue. Hayden² revised Rosenow's procedure by adding crushed marble to the medium and reported favorable growth of dental pathogens. Brain Heart Infusion is a modification of the media described by Rosenow¹ and Hayden² in which infusion from calf brains has replaced the brain tissue, and disodium phosphate has replaced the calcium carbonate.

Infusion from beef heart, calf brains and Proteose Peptone provide nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion media. Dextrose is a carbon energy source to facilitate organism growth, sodium chloride maintains osmotic balance in the medium, and disodium phosphate is a buffering agent.

Bacto Brain Heart Infusion, Porcine was developed as an alternative to the classical Brain Heart Infusion formula, and replaces calf brains and beef heart with pork brains and heart. BHI, Porcine was formulated with no bovine components to minimize Bovine Spongiform Encephalopathy (BSE) risk.

Infusion from pork brains, infusion from pork heart, and Pork Peptone No. 2 provide nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion, Porcine. Dextrose is a carbon energy source to facilitate organism growth, sodium chloride maintains osmotic balance in the medium, and disodium phosphate is a buffering agent.

Applications

Brain Heart Infusion media are specified in several standard methods references for food testing.³⁻⁵ *Standard Methods for the Examination of Water and Wastewater* recommends Brain Heart Infusion media in tests for the verification of fecal streptococci.⁶ Brain Heart Infusion is listed by the National Committee for Clinical Laboratory Standards (NCCLS) as a medium for use in the preparation of microdilution trays for antimicrobial susceptibility testing by the broth microdilution procedure.⁷

BHI broth has been used in various microbiological studies. BHI is recommended for growth of most ATCC™ strains of *Pasteurella multocida* and has been cited in many studies on *P. multocida* fowl cholera vaccines. [Duffy et al.](#)⁸ used BHI as a fermentation medium for pH studies on *Escherichia coli* O157:H7. [Tan et al.](#)⁹ utilized BHI to grow *Fusobacterium necrophorum* for leukotoxin production studies. Likewise, [Van Tassell et al.](#)¹⁰ made enterotoxin preparations by growing *Bacteroides fragilis* in BHI.

BHI, Porcine was developed for pharmaceutical and vaccine production and can replace the traditional BHI depending on organism and production application.

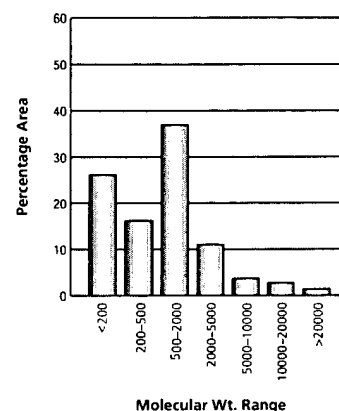
Physical Characteristics

Bacto Brain Heart Infusion is a light tan, free-flowing, homogeneous powder.

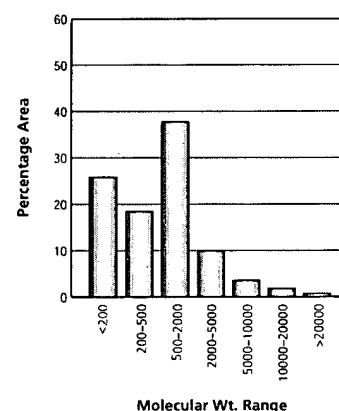
Bacto Brain Heart Infusion, Porcine is a light tan, free-flowing, homogeneous powder.

Molecular Weights

Bacto Brain Heart Infusion



Bacto Brain Heart Infusion, Porcine



Formula* Per Liter

Bacto™ Brain Heart Infusion

Calf Brains, Infusion from 200 g.....7.7 g
Beef Heart, Infusion from 250 g.....9.8 g
Bacto Proteose Peptone.....10.0 g
Dextrose.....2.0 g
Sodium Chloride.....5.0 g
Disodium Phosphate.....2.5 g
Final pH 7.4 ± 0.2 at 25°C

Bacto™ Brain Heart Infusion, Porcine

Pork Brains, Infusion from 200 g.....7.7 g
Pork Heart, Infusion from 250 g.....9.8 g
Bacto Pork Peptone No. 2.....10.0 g
Dextrose.....2.0 g
Sodium Chloride.....5.0 g
Disodium Phosphate.....2.5 g
Final pH 7.4 ± 0.2 at 25°C

*Adjusted and/or supplemented as required to meet performance criteria.

Availability

Bacto™ Brain Heart Infusion 237400, 100 g

Bacto™ Brain Heart Infusion 237500, 500 g

Bacto™ Brain Heart Infusion 237200, 2 kg

Bacto™ Brain Heart Infusion 237300, 10 kg

Bacto™ Brain Heart Infusion, Porcine 256120, 500 g

Bacto™ Brain Heart Infusion, Porcine 256110, 10 kg

References

1. Rosenow. 1919. Studies on elective localization. J. Dent. Res. 1:205-249.
2. Hayden. 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Intern. Med. 32:828-849.
3. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, MD.
4. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC.
6. Clesceri, Greenberg and Eaton (ed.). 1998. Membrane filter techniques, 9-72-74. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, DC.
7. National Committee for Clinical Laboratory Standards. 1997. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 4th ed. Approved standard M11-A4. National Committee for Clinical Laboratory Standards, Wayne, PA.
8. Duffy, Riordan, Sheridan, Call, Whiting, Blair and McDowell. 2000. Effect of pH on survival, thermotolerance, and verotoxin production of *Escherichia coli* O157:H7 during simulated fermentation and storage. J. Food Prot. 63:12-18.
9. Tan, Nagaraja and Chengappa. 1992. Factors affecting leukotoxin activity of *Fusobacterium necrophorum*. Vet. Microbiol. 32:15-28.
10. Van Tassell, Lyerly and Wilkins. 1992. Purification and characterization of an enterotoxin from *Bacteroides fragilis*. Infect. Immun. 60:1343-1350.

GELYSATE™ PEPTONE

Product Description

Gelysate™ Peptone is a pancreatic digest of gelatin. Gelatin is extracted from collagen, which is the fibrous protein in bone, cartilage and connective tissue. Gelatin hydrolysate is high in proline residues.¹ Gelysate Peptone is deficient in carbohydrates and is characterized by low cystine, methionine and tryptophan content.

Applications

Gelysate Peptone should be used for cultures requiring low carbohydrates, cystine, and tryptophan levels in cell culture and bacterial fermentation.

Physical Characteristics

Gelysate Peptone is a tan, free-flowing, homogeneous powder.

Availability

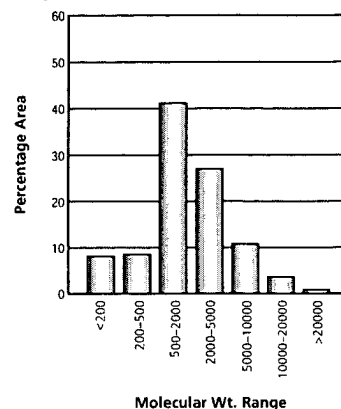
Gelysate™ Peptone 211870, 454 g

Reference

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. In Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.

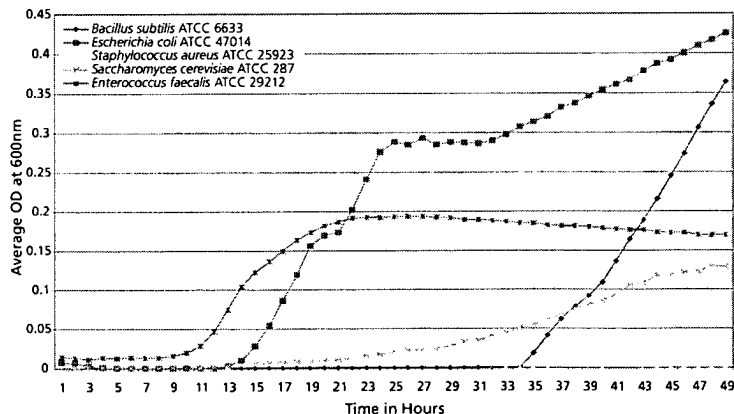
Molecular Weights

Gelysate Peptone



Growth Curve

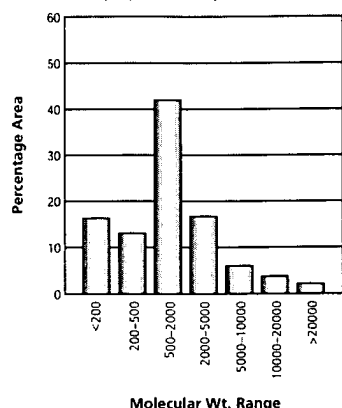
1% Gelysate Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



BACTO™ NEOPEPTONE PEPTONE

Molecular Weights

Bacto Neopeptone Peptide



Product Description

Bacto™ Neopeptone Peptide is an enzymatic digest of protein. Neopeptone contains a wide variety of peptide sizes in combination with vitamins, nucleotides and minerals.

Applications

Neopeptone is recommended for use in media for detection of fungi.¹ Apodaca and McKerrow² used Neopeptone for the cultivation of *Trichophyton rubrum* for study of its proteolytic activity. Neopeptone has been cited as a component of culture media used for cultivation of human pathogens, notably, *Bordetella pertussis* and group A streptococci.

Neopeptone has also been reported to provide nutrients for support of spirochetes and protozoa. Wyss et al.³ used Neopeptone as a component of a medium for cultivation of *Treponema maltophilum* sp. nov., a fastidious oral anaerobe. Ifediba and Vanderberg⁴ reported that Neopeptone, in addition to calf serum, was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Cushion and Ebbets⁵ utilized Neopeptone in their investigations of various media for cultivating *Pneumocystis carinii* without feeder cells; optimal replication of *P. carinii* separated from host fungi cells was observed in media with Neopeptone and N-acetylglucosamine at low pH.

Physical Characteristics

Bacto Neopeptone appears as tan, free-flowing, granules.

Availability

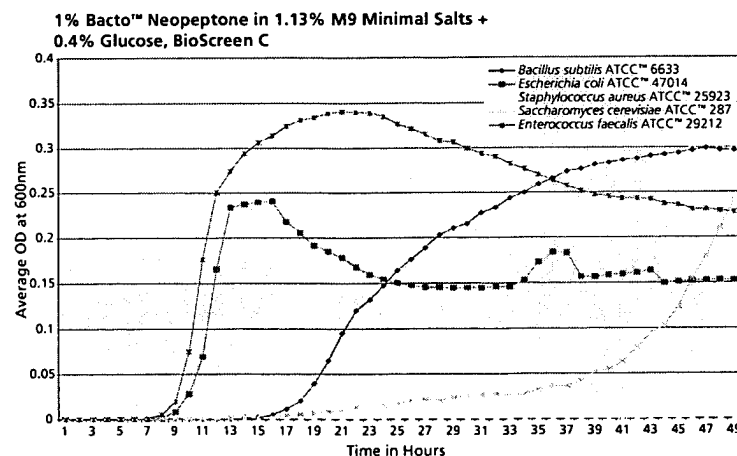
Bacto™ Neopeptone 211681, 500 g

Bacto™ Neopeptone 211680, 10 kg

References

1. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed., 9-131-137. American Public Health Association, Washington, DC.
2. Apodaca and McKerrow. 1990. Expression of proteolytic activity by cultures of *Trichophyton rubrum*. J. Med. Vet. Mycol. 28:159-171.
3. Wyss, Choi, Schupbach, Guggenheim and Gobel. 1995. *Treponema maltophilum* sp. nov., a small oral spirochete isolated from human periodontal lesions. Int. J. Syst. Bacteriol. 46:745-752.
4. Ifediba and Vanderberg. 1980. Peptones and calf serum as a replacement for human serum in the cultivation of *Plasmodium falciparum*. J. Parasitol. 66:236-239.
5. Cushion and Ebbets. 1990. Growth and metabolism of *Pneumocystis carinii* in axenic culture. J. Clin. Microbiol. 28:1385-1394.

Growth Curve



BACTO™ PEPTONE

BITEK™ PEPTONE

Product Description

Bacto™ Peptone is an enzymatic digest of animal protein. Bacto Peptone was first introduced in 1914 and became the standard Peptone for the preparation of bacteriological culture media. The nutritive value of Bacto Peptone is largely dependent on the amino acid content that supplies essential nitrogen. Bacto Peptone contains only a negligible quantity of proteoses and more complex constituents.

BiTek™ Peptone is an enzymatic digest of animal protein. BiTek Peptone has growth properties similar to Bacto Peptone.

Applications

Bacto Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi. For example, [Iwanaga et al.](#)¹ utilized Bacto Peptone for production of cholera toxin by *Vibrio cholerae* O1 El Tor. [Benkerroum et al.](#)² reported using Bacto Peptone in a selective medium developed for isolating *Leuconostoc* spp. from food samples. Bacto Peptone was used in a culture medium for two anaerobic, extremely thermophilic Archaea, *Thermococcus celer* and *Pyrococcus woesei*, by [Blamey et al.](#)³

Bacto Peptone has also been utilized as a nitrogen source in cell culture media formulations. [Taylor et al.](#)⁴ used Bacto Peptone to supplement serum-free medium for several mammalian cell lines and reported the solubility of Bacto Peptone as very good at 10 g/100 mL water. Sakoda and Fukusho⁵ also utilized Bacto Peptone in serum-free culture for maintaining porcine kidney epithelial cells. Bacto Peptone is also useful as a supplement in cell culture with serum.

Researchers uncovered estrogenic activity associated with Bacto Peptone when including the peptone in medium for culture of yeast; the estrone contained in Bacto Peptone was converted to estradiol by *Saccharomyces cerevisiae*. These findings suggest that adding estrogens to a medium containing Bacto Peptone for studies of estradiol production by yeast may confound results.^{6,7}

BiTek Peptone is used as a nitrogen source in microbiological culture media. BiTek Peptone offers the same lot-to-lot consistency and similar growth characteristics to Bacto Peptone.

Physical Characteristics

Bacto Peptone appears as tan, free-flowing, granules.

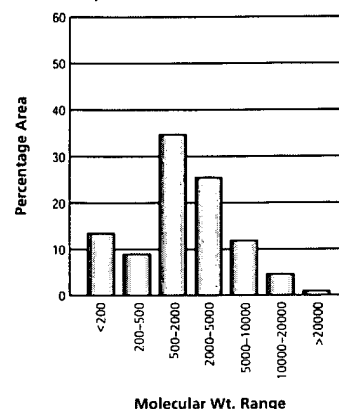
BiTek Peptone is a light beige, free-flowing, homogeneous powder.

References

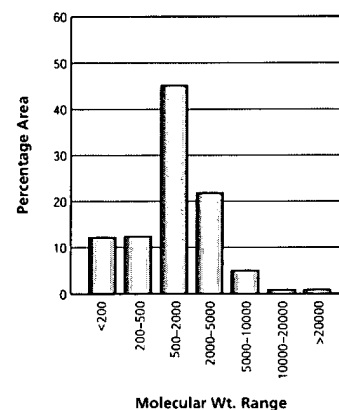
1. Iwanaga, Yamamoto, Higa, Ichinose, Nakasone and Tanabe. 1986. Culture conditions for stimulating cholera toxin production by *Vibrio cholerae* O1 El Tor. *Microbiol. Immunol.* 30:1075-1083.
2. Benkerroum, Misbah, Sandine and Elaraki. 1993. Development and use of a selective medium for isolation of *Leuconostoc* spp. from vegetables and dairy products. *Appl. Environ. Microbiol.* 59:607-609.
3. Blamey, Chiong, Lopez and Smith. 1999. Optimization of the growth conditions of the extremely thermophilic microorganisms *Thermococcus celer* and *Pyrococcus woesei*. *J. Microbiol. Methods* 38:169-175.
4. Taylor, Dworkin, Pumper and Evans. 1972. Biological efficacy of several commercially available peptones for mammalian cells in culture. *Exp. Cell Res.* 74:275-279.
5. Sakoda and Fukusho. 1998. Establishment and characterization of a porcine kidney cell line, FS-L3, which forms unique multicellular domes in serum-free culture. *In Vitro Cell. Dev. Biol. Anim.* 34:53-57.
6. Feldman and Krishnan. 1995. Estrogens in unexpected places: possible implications for researchers and consumers. *Environ. Health Perspect.* 103 Suppl 7:129-133.
7. Miller, Bottema, Stathis, Tokes and Feldman. 1986. Unexpected presence of estrogens in culture medium supplements: subsequent metabolism by the yeast *Saccharomyces cerevisiae*. *Endocrinology* 119:1362-1369.

Molecular Weights

Bacto Peptone



BiTek Peptone



Availability

Bacto™ Peptone 211840, 100 g

Bacto™ Peptone 211677, 500 g

Bacto™ Peptone 211820, 2 kg

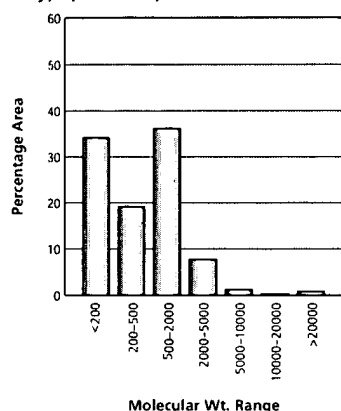
Bacto™ Peptone 211830, 10 kg

BiTek™ Peptone 254820, 10 kg

POLYPEPTONE™ PEPTONE

Molecular Weights

Polypeptide Peptide



Product Description

Polypeptide™ Peptide is a mixture of peptones made up of equal parts of pancreatic digest of casein and peptic digest of animal tissue. Polypeptide Peptide includes the high content of amino acids and small polypeptides characteristic of pancreatic digest of casein and the larger polypeptides characteristic of peptic digest of animal tissue.

Applications

Researchers have found Polypeptide Peptide to meet nutritional requirements of various bacteria, fungi and mammalian cells, where a single source of casein or meat peptones has been unsatisfactory. Polypeptide Peptide has been utilized in culture media for the production of trypsin inhibitor by *Cephalosporium* sp.;¹ the production of bacterial cellulose by *Acetobacter* sp. A9;² production of succinic acid from whey by *Anaerobiospirillum succiniciproducens*;³ mass production of luciferase-bacterial magnetic particles by recombinant *Magnetospirillum magneticum* AMB-1;⁴ and the production of a novel tumor-killing factor by human macrophage-monocyte hybridomas.⁵

Physical Characteristics

Polypeptide Peptide is a yellow to tan, free-flowing, homogeneous powder.

Availability

Polypeptide™ Peptide 211910, 454 g

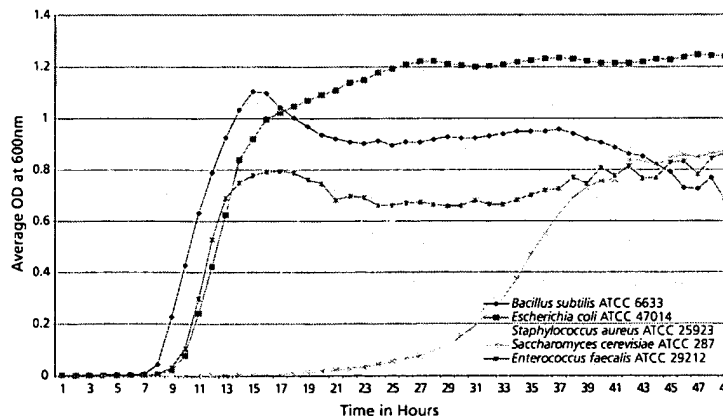
Polypeptide™ Peptide 297108, 10 kg

References

1. Tsuchiya and Kimura. 1978. Production of trypsin inhibitor by a *Cephalosporium* sp. Appl. Environ. Microbiol. 35:631-635.
2. Son, Heo, Kim and Lee. 2001. Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures. Biotechnol. Appl. Biochem. 33(Pt 1):1-5.
3. Lee, Lee, Kwon, Lee and Chang. 2000. Batch and continuous cultivation of *Anaerobiospirillum succiniciproducens* for the production of succinic acid from whey. Appl. Microbiol. Biotechnol. 54:23-27.
4. Yang, Takeyama, Tanaka and Matsunaga. 2001. Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-bacterial magnetic particle complex by a recombinant *Magnetospirillum magneticum* AMB-1. Enzyme Microbiol. Technol. 29:13-19.
5. Taniyama, Yoshida and Furuta. 1988. Demonstration of a novel tumor-killing factor secreted from human macrophage-monocyte hybridomas. J. Immunol. 141:4061-4065.

Growth Curve

1% Polypeptide Peptide in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



BACTO™ PROTEOSE PEPTONE

BITEK™ PROTEOSE PEPTONE

BACTO™ PROTEOSE PEPTONE NO. 2

BACTO™ PROTEOSE PEPTONE NO. 3

BITEK™ PROTEOSE PEPTONE NO. 3

BACTO™ PROTEOSE PEPTONE NO. 4

Product Description

The Bacto™ Proteose Peptones are enzymatic digests of protein. Studies of peptic digests of animal tissue prepared under varying digestion parameters led to the development of Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3. Data accumulated during these studies demonstrated that no one peptone is the most suitable nitrogen source for every microbiological application. Bacto Proteose Peptone No. 4 is a spray-dried version of Bacto Proteose Peptone.

BiTek™ Proteose Peptone and BiTek Proteose Peptone No. 3 are enzymatic digests of protein, developed to offer alternatives to the Bacto Proteose Peptones for scale-up to production applications.

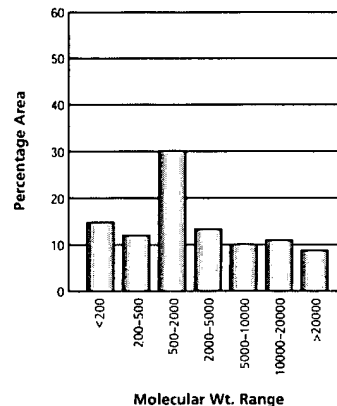
Applications

Bacto Proteose Peptone is used in preparing microbiological culture media and in producing bacterial toxins. Bacto Proteose Peptone was originally developed to produce a diphtheria toxin of high and uniform potency from cultures of *Corynebacterium diphtheriae*. Studies support the use of Proteose Peptone for production of diphtheria toxin, toxin-antitoxin mixtures and toxoid.^{1,2} Proteose Peptone is also valuable in the production of other bacterial toxins: *Clostridium botulinum* toxin;³ toxin from *Clostridium perfringens*;⁴ toxin of hemolytic streptococci;⁵ pneumococcus toxin;⁶ and toxin from *Salmonella pullorum* (*Salmonella choleraesuis* subsp. *choleraesuis*).⁷

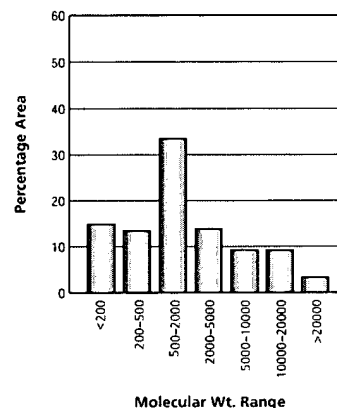
Many factors account for the suitability of Proteose Peptone for the culture of fastidious pathogens, including the nitrogen components, buffering range and the high content

Molecular Weights

Bacto Proteose Peptone

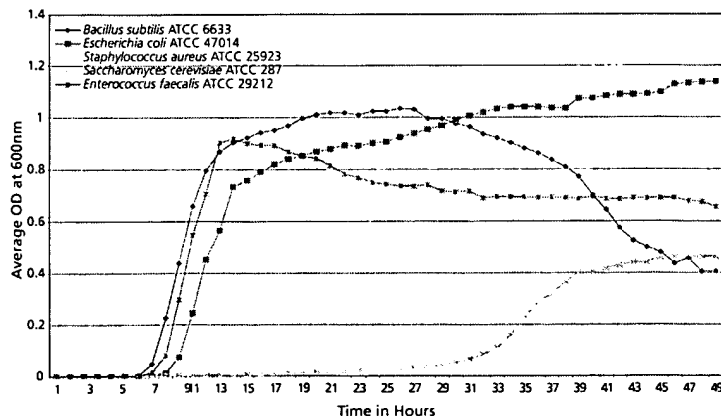


BiTek Proteose Peptone



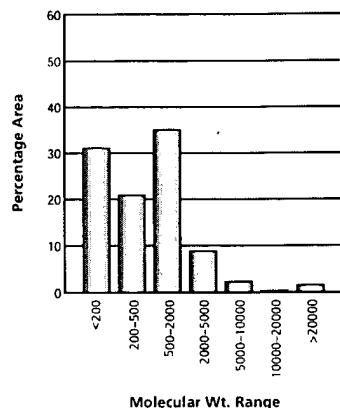
Growth Curve

1% Bacto Proteose Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C

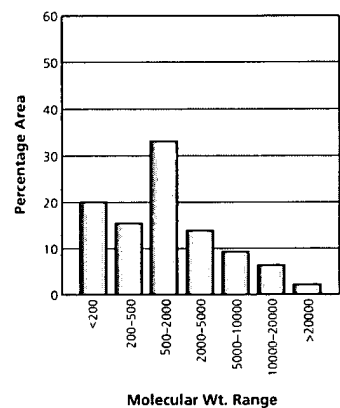


Molecular Weights

Bacto Proteose Peptone No. 2



Bacto Proteose Peptone No. 3

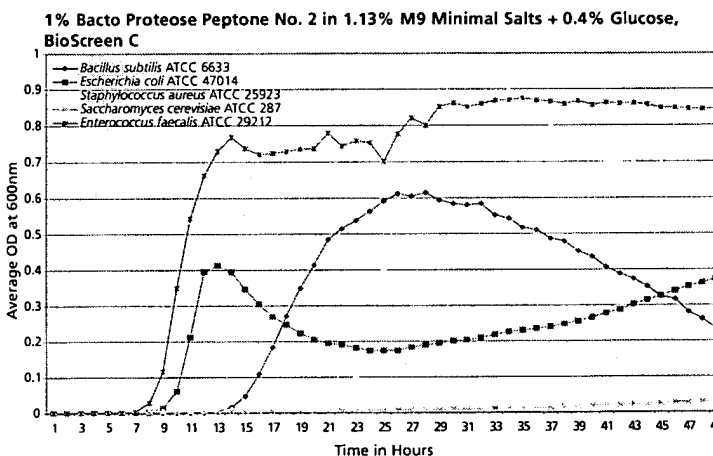


of proteoses. These elements create an environment beneficial to the maintenance of virulence and the elaboration of bacterial by-products, thus stock cultures are well preserved on media containing Bacto Proteose Peptone.

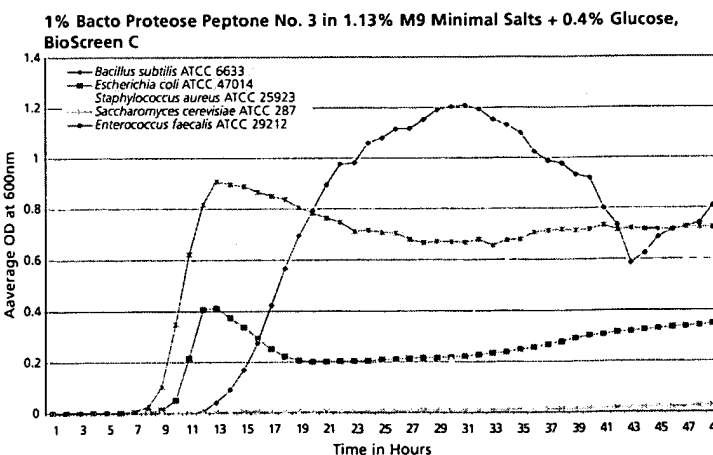
Bacto™ Proteose Peptone may be used in culture medium for a variety of applications, including production of substances from the culture of bacteria, fungi and mammalian cells. Proteose Peptone has been utilized in a medium for producing glycosidases from *Bacteroides fragilis*,⁸ and to stimulate amyloglucosidase production by *Aspergillus* sp.⁹ It has been used to cultivate halophilic bacteria isolated from soil in Egypt for production of polymers.¹⁰ Jan et al.¹¹ reported that Proteose Peptone as supplementation to defined medium resulted in significant increases in cell number and specific monoclonal antibody production in batch culture system. Proteose Peptone has also been used to provide nutrients for axenic culture of amoeba.¹²

BiTek Proteose Peptone was developed to provide an alternative product to Bacto Peptone with growth characteristics similar to Bacto Proteose Peptone.

Growth Curve



Growth Curve



Bacto™ Proteose Peptone No. 2 is used in preparing microbiological culture media. It was originally developed for use in media for the production of diphtheria toxin. Bunney and Thomas¹³ reported good yield of diphtheria toxin with Proteose Peptone No. 2 in a simple peptone-sugar-sodium acetate medium.

Bacto™ Proteose Peptone No. 3 is used in preparing microbiological culture media. It is a modification of Proteose Peptone adapted for use in the preparation of chocolate agar for propagation of *Neisseria* species and chocolate tellurite agar for *Corynebacterium diphtheriae*. While investigating the nutritional values of the Proteose Peptones, Difco Laboratories found that Proteose Peptone No. 3 provides superior nutrition for fastidious microorganisms. It supports growth of streptococci, staphylococci, pneumococci, gonococci and other organisms that require a highly nutritious substrate. For example, Ifediba and Vanderberg¹⁴ report that Proteose Peptone No. 3 in addition to calf serum was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Recently, because of the benefit of lower endotoxin levels, cell culture manufacturers have found significant yield improvements in using Proteose Peptone No. 3.

BiTek Proteose Peptone No. 3 was developed to provide an alternative product to Bacto Proteose Peptone No. 3 with growth characteristics similar to Bacto Proteose Peptone No. 3.

Bacto Proteose Peptone No. 4 is a spray-dried version of Bacto Proteose Peptone. It offers the same beneficial nutrients as Proteose Peptone for growth promotion and toxin production with a wide range of fastidious microorganisms.

Physical Characteristics

Bacto Proteose Peptone appears as tan, free-flowing granules.

BiTek Proteose Peptone is a tan, free-flowing, homogeneous powder.

Bacto Proteose Peptone No. 2 appears as tan, free-flowing granules.

Bacto Proteose Peptone No. 3 appears as golden tan, free-flowing granules.

BiTek Proteose Peptone No. 3 is a tan, free-flowing, homogeneous powder.

Bacto Proteose Peptone No. 4 is a light beige, free-flowing, homogeneous powder.

Availability

Bacto™ Proteose Peptone 211684, 500 g

Bacto™ Proteose Peptone 212010, 10 kg

BiTek™ Proteose Peptone 253310, 10 kg

Bacto™ Proteose Peptone No. 2 212120, 500 g

Bacto™ Proteose Peptone No. 2 212110, 10 kg

Bacto™ Proteose Peptone No. 3 211693, 500 g

Bacto™ Proteose Peptone No. 3 212220, 2 kg

Bacto™ Proteose Peptone No. 3 212230, 10 kg

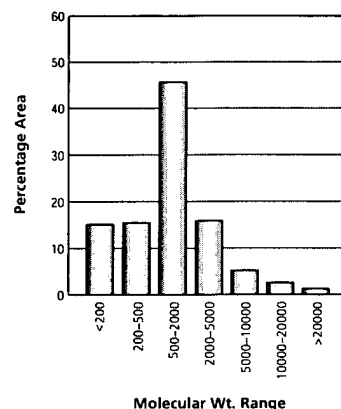
Bacto™ Proteose Peptone No. 3 211692, 50 kg

BiTek™ Proteose Peptone No. 3 253720, 25 kg

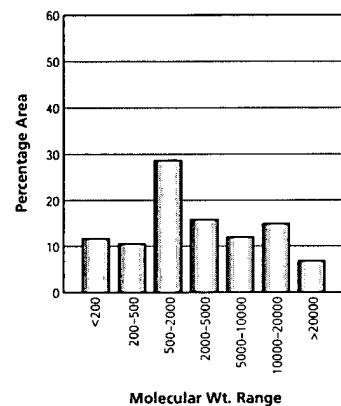
Bacto™ Proteose Peptone No. 4 211715, 10 kg

Molecular Weights

BiTek Proteose Peptone No. 3



Bacto Proteose Peptone No. 4



References

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5. Kirkbride and Wheeler. 1926. Studies of the toxins of the hemolytic streptococci associated with scarlet fever. *J. Immunol.* 11:477-497.
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7. Hanks and Rettger. 1931. Bacterial endotoxin; search for a specific intracellular toxin in *S. pullorum*. *J. Immunol.* 22:283-314.
8. Berg, Nord and Wadstrom. 1978. Formation of glycosidases in batch and continuous culture of *Bacteroides fragilis*. *Appl. Environ. Microbiol.* 35:269-273.
9. Mamo and Gessesse. 1999. Production of raw-starch digesting amyloglucosidase by *Aspergillus* sp. GP-21 in solid state fermentation. *J. Ind. Microbiol. Biotechnol.* 22:622-626.
10. Hezayen, Rehm, Eberhardt and Steinbuechel. 2000. Polymer production by two newly isolated extremely halophilic archaea: application of a novel corrosion-resistant bioreactor. *Appl. Microbiol. Biotechnol.* 54:319-325.
11. Jan, Jones, Emery and Al-Rubeai. 1994. Peptone, a low-cost growth-promoting nutrient for intensive animal cell culture. *Cytotechnol.* 16:17-26.
12. Shukla, Kaul and Mehlotra. 1989. Development of improved media for axenic cultivation of *Acanthamoeba culbertsoni*, Singh and Das 1970. *Indian J. Exp. Biol.* 27:785-791.
13. Bunney and Thomas. 1936. Diphtheria toxin-production on broths made from dried complete media. *J. Immunol.* 31:95-102.
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THIOTONE™ E PEPTONE

Product Description

Thiotone™ E Peptone is an enzymatic digest of animal tissue. Thiotone E Peptone contains a wide range of peptide sizes, including the large molecular weight peptides which support fastidious organisms.

Applications

Thiotone E Peptone is a source of nitrogen, amino acids and vitamins in microbiological culture media. It has been recommended for use in blood agar formulae for hemolysis studies with pneumococci and streptococci. Thiotone E Peptone is high in sulfur amino acids and can be used in media to detect hydrogen sulfide production. Tortora¹ utilized Thiotone E Peptone as the nitrogen source in a medium promoting sporulation of *Clostridium perfringens* strains. Thiotone E Peptone is recommended for use in media for testing water samples for coliforms.² Kwinn³ utilized Thiotone E Peptone as a supplement to her medium for *Corynebacterium glutamicum* to make the cells electrocompetent for transformations. Thiotone E Peptone has also been cited as an ingredient in media for non-bacterial organisms; Thiotone E Peptone is used in Modified HL5 Medium, one of the main media used for culturing the cellular slime mold *Dictyostelium discoideum*.

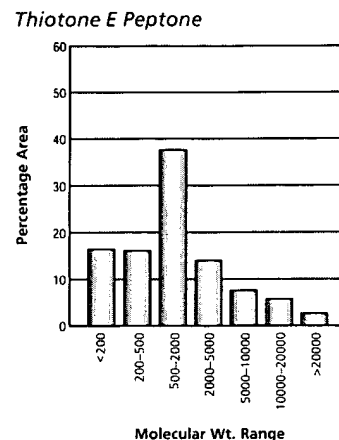
Physical Characteristics

Thiotone E Peptone is a tan, free-flowing, homogeneous powder.

Availability

Thiotone™ E Peptone 212302, 500 g

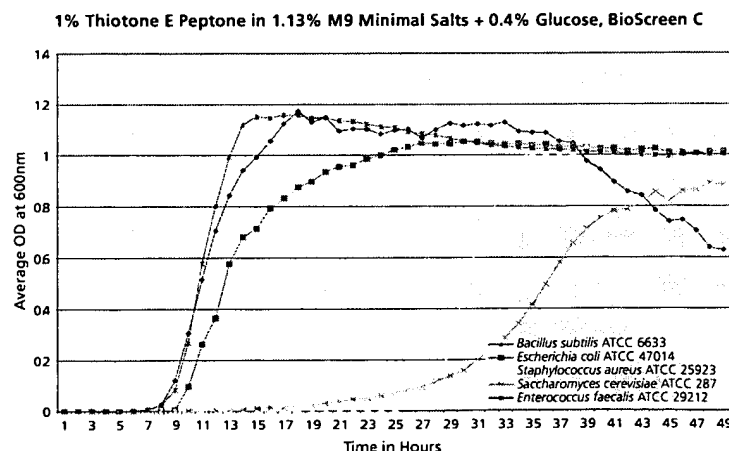
Molecular Weights



References

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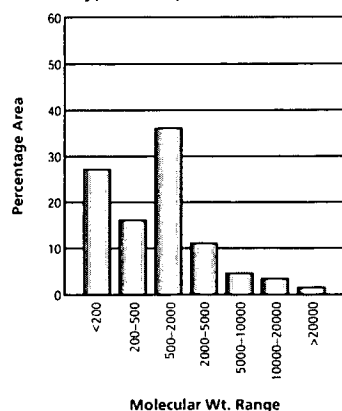
Growth Curve



BACTO™ TRYPTOSE PEPTONE

Molecular Weights

Bacto Tryptose Peptone



Product Description

Bacto™ Tryptose Peptone is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process of Tryptose results in assorted peptides of higher molecular weight suitable for long-chain amino acid requirements.

Applications

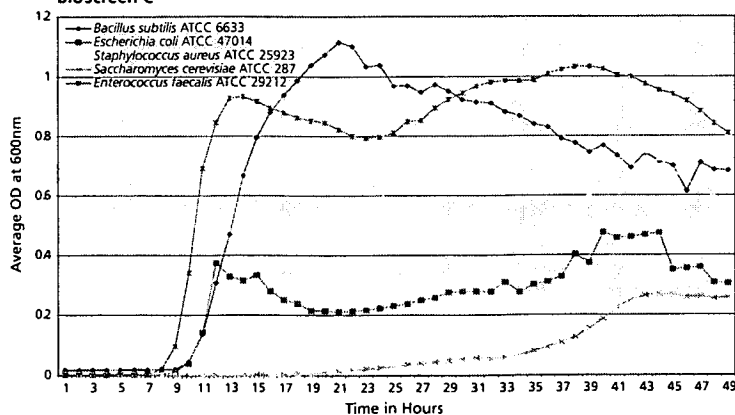
Bacto Tryptose Peptone was originally developed as a peptone particularly adapted to the growth requirements of *Brucella*. Tryptose is very useful for cultivation of streptococci, pneumococci, meningococci and other fastidious organisms, and was found to be superior to meat infusion peptone media previously used for these organisms.^{1,2} Mobley et al.³ reported that Tryptose Broth was the preferred medium for strains of *Bordetella bronchiseptica* in studies of phosphatase activity.

Tryptose has been reported as beneficial for cell culture applications. Litwin⁴ found Tryptose suited to supplementing a serum-free medium for growing human diploid fibroblasts. Vaughn and Fan⁵ established that Tryptose provided free amino acids necessary for growth of *Spodoptera frugiperda* and *Lymantria dispar* insect cell lines. Tryptose Peptone is often used as a biomass enhancer for recombinant *Escherichia coli* production.

Tryptose is the major ingredient and only peptone in the formulation, Tryptose Phosphate Broth, an often-used medium for various culture applications. Hata and Kojima⁶ have shown Tryptose Phosphate Broth (TPB) to be a useful supplement in culturing the nematode, *Angiostrongylus cantonensis*, *in vitro*. TPB was also reported as a supplement to a medium for cultivating a protozoan parasite, which parasitizes vectors of Chagas' disease, on its insect cell host.⁷ *Spodoptera frugiperda*, a cotton pest in Argentina⁸ and several tick cell lines have also been grown using a TPB supplemented medium.⁹ Tryptose Phosphate Broth has been reported as a suitable supplement for growth of baby hamster kidney cells¹⁰ and porcine kidney cells.¹¹

Growth Curve

1% Bacto Tryptose Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Physical Characteristics

Bacto Tryptose Peptone appears as tan, free-flowing granules.

Availability

Bacto™ Tryptose Peptone 211713, 500 g

Bacto™ Tryptose Peptone 211709, 10 kg

References

1. Casman, 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. *J. Bacteriol.* 43:33.
2. Casman, 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. *Am. J. Clin. Pathol.* 17:281-289.
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MEAT PEPTONES TYPICAL ANALYSES

Product Name	Total Nitrogen (%)	Amino Nitrogen (%)	AN/TN	Total Carbohydrate (mg/g)	Ash (%)	Loss on Drying (%)	NaCl (%)	pH (2% Solution)	Calcium (ug/g)	Magnesium (ug/g)	Potassium (ug/g)	Sodium (ug/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% Free)	Alanine (% Total)	Arginine (% Free)	Arginine (% Total)	Asparagine (% Free)	Aspartic Acid (% Free)	Aspartic Acid (% Total)	Cystine (% Free)
Beef Extract, Desiccated, Bacto™	13.9	2.0	0.14	9.80	7.7	1.8	1.7	6.9	53	92	31423	21645	1.62	0.70	0.43	1.1	7.1	1.3	4.2	0.1	0.3	2.4	0.0
Beef Extract, Powder	12.4	2.3	0.19	56.10	9.3	3.5	0.3	6.9	264	285	28793	18510	0.00	0.53	3.22	1.8	4.0	2.8	2.8	0.6	0.6	5.5	0.2
Brain Heart Infusion, Bacto	10.2	3.9	0.38	84.43	22.9	1.5	3.2	7.3	103	394	19810	72870	9.05	0.25	0.22	1.3	6.5	1.1	3.2	0.4	0.7	3.9	0.5
Brain Heart Infusion, Porcine, Bacto	9.2	4.0	0.43	67.07	22.4	1.8	2.2	7.4	366	374	19020	80930	8.77	0.27	0.25	1.4	6.6	0.9	3.5	0.4	0.9	3.8	0.5
Gelysate™ Peptone	17.0	2.9	0.17	11.58	3.8	4.9	0.2	6.9	381	150	656	11090	0.00	1.66	0.18	0.8	8.8	3.1	6.3	0.1	0.1	4.7	0.3
Neopeptone, Bacto	13.6	3.2	0.20	13.13	6.9	4.0	1.4	7.4	77	28	8945	36313	0.48	0.45	2.59	0.5	4.3	0.5	2.6	0.2	0.3	4.2	0.4
Peptone, Bacto	15.4	3.5	0.20	6.29	3.8	2.7	1.7	7.1	30	17	2487	18127	0.90	0.32	0.40	1.2	9.2	2.8	5.8	0.3	0.3	5.0	0.0
Peptone, BiTek™	16.4	3.0	0.18	11.29	3.4	2.0	0.0	6.9	400	150	840	10530	0.01	1.82	0.07	0.7	8.3	3.7	6.3	0.1	0.1	3.9	0.7
Polypeptone™ Peptone	13.1	5.2	0.40	8.06	9.7	4.9	2.7	7.3	271	342	7340	44257	1.00	0.40	3.40	1.2	4.1	2.4	3.3	0.4	0.4	6.1	0.3
Proteose Peptone, Bacto	14.3	2.8	0.20	12.02	7.8	3.0	4.9	6.7	120	261	9123	29730	2.65	0.19	0.64	0.5	6.0	0.4	4.7	0.1	0.4	5.3	0.4
Proteose Peptone, BiTek	13.1	3.1	0.24	10.30	13.1	4.8	10.3	6.8	219	680	7390	44750	4.93	1.01	0.94	0.8	7.0	0.4	4.4	0.1	0.6	3.9	0.4
Proteose Peptone No. 2, Bacto	12.9	5.0	0.39	18.07	12.1	3.5	7.1	7.3	151	212	13313	47610	3.86	0.38	1.88	1.6	5.2	1.4	4.1	0.5	1.1	5.5	1.0
Proteose Peptone No. 3, Bacto	13.4	3.7	0.28	17.94	10.5	2.3	6.6	7.4	132	103	13160	38113	2.54	0.37	1.51	0.90	5.2	0.8	4.3	0.3	0.6	5.1	0.6
Proteose Peptone No. 3, BiTek	12.8	3.1	0.24	12.35	13.1	1.3	12.5	6.7	129	214	8682	50153	9.40	0.17	1.22	0.8	6.4	0.8	5.1	0.1	0.7	5.7	1.2
Proteose Peptone No. 4, Bacto	14.3	2.7	0.19	12.17	7.8	3.3	3.9	7.0	169	280	9109	35280	2.63	0.34	0.72	0.5	6.5	0.4	4.6	0.1	0.3	4.4	0.3
Thiotone™ E Peptone	13.4	3.4	0.25	30.71	11.4	4.8	8.2	6.7	196	270	9629	46683	4.17	0.81	0.65	1.0	6.7	0.9	4.3	0.1	0.9	4.4	0.6
Tryptose, Bacto	13.3	4.5	0.34	10.56	8.8	3.2	3.2	7.3	191	110	9292	37740	1.61	0.23	2.05	1.2	4.3	1.9	3.5	0.4	0.5	5.1	0.4

* = Partially destroyed during hydrolysis

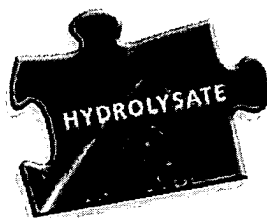
0.0 = Below limits of detection

For test methods see Definition of Methods section on page G1.

Free Amino Acids / Total Amino Acids

Glutamic Acid (% Free)	Glutamic Acid (% Total)	Glutamine (% Free)	Glycine (% Free)	Glycine (% Total)	Histidine (% Free)	Histidine (% Total)	Isoleucine (% Free)	Isoleucine (% Total)	Leucine (% Free)	Leucine (% Total)	Lysine (% Free)	Lysine (% Total)	Methionine (% Free)*	Methionine (% Total)	Phenylalanine (% Free)	Phenylalanine (% Total)	Proline (% Free)	Proline (% Total)	Serine (% Free)	Serine (% Total)*	Threonine (% Free)	Threonine (% Total)	Tryptophan (% Free)	Tyrosine (% Free)	Tyrosine (% Total)	Valine (% Free)	Valine (% Total)
0.6	6.4	0.0	1.0	8.2	0.1	1.4	0.2	1.3	0.4	2.8	0.6	2.5	0.3	0.7	0.2	1.5	0.4	7.2	0.3	0.3	0.2	0.4	0.2	0.3	0.8	0.2	2.0
2.5	14.6	0.1	0.5	2.3	0.4	2.1	1.3	5.1	3.8	7.2	4.0	5.7	0.8	1.6	2.5	5.0	0.3	5.7	0.8	2.1	0.6	1.8	0.7	0.6	1.5	1.4	5.4
1.4	6.4	0.1	0.4	3.7	0.2	1.2	0.7	2.3	2.5	4.4	1.6	4.2	0.8	1.0	1.6	2.3	0.2	3.4	0.6	*	0.5	0.7	0.3	0.8	1.2	0.7	3.1
1.7	5.9	0.1	0.4	4.3	0.2	0.9	0.6	2.1	2.1	4.1	1.4	3.9	0.8	0.9	1.4	2.1	0.3	3.2	0.6	*	0.4	0.6	0.3	0.8	1.2	0.7	2.8
0.2	7.9	0.1	0.5	16.8	0.3	1.0	0.5	1.6	0.9	3.2	2.0	3.3	0.3	0.8	1.1	2.4	0.1	9.7	0.2	1.8	0.1	0.9	0.0	0.5	0.6	0.3	2.3
0.6	7.4	0.0	0.2	3.4	0.1	1.2	0.3	2.3	1.6	4.6	0.8	4.0	0.5	1.0	1.3	2.7	0.1	4.7	0.3	0.8	0.2	0.9	0.3	0.8	2.2	0.3	2.9
0.7	8.1	0.0	0.7	15.9	0.2	0.8	0.6	2.1	1.6	3.8	2.2	3.4	0.3	0.7	1.4	2.8	0.3	8.8	0.4	1.5	0.3	1.1	0.3	0.5	0.6	0.7	2.8
0.2	6.3	0.1	0.5	12.7	0.3	0.8	0.4	1.2	1.1	2.4	1.8	0.2	0.5	0.8	0.9	1.5	0.1	7.1	0.2	0.4	0.0	0.6	0.0	0.6	0.6	0.4	2.0
0.9	12.6	0.1	0.5	3.0	0.4	2.1	1.1	3.8	3.9	6.2	3.6	6.2	1.0	1.9	2.4	3.6	0.3	5.4	0.7	2.1	0.7	1.9	0.6	0.7	1.6	1.3	4.7
0.7	8.4	0.0	0.2	8.2	0.1	1.3	0.3	3.3	1.4	5.7	0.8	4.2	0.3	1.4	1.0	3.6	0.1	4.6	0.2	1.7	0.2	1.5	0.1	0.6	1.8	0.2	3.7
0.4	6.3	0.1	0.4	7.3	0.1	0.8	0.4	2.0	1.4	4.2	0.9	3.4	0.6	1.0	1.1	2.3	0.1	6.3	0.2	0.3	0.1	0.7	0.1	0.5	1.2	0.4	2.8
1.8	7.5	0.1	0.9	6.2	0.3	1.3	1.1	3.7	3.3	6.2	2.5	4.2	0.8	1.2	2.2	3.9	0.5	3.8	0.8	1.9	0.6	1.7	0.5	0.7	1.3	1.0	4.0
1.2	8.0	0.0	0.4	6.5	0.1	1.3	0.6	3.2	2.3	5.6	1.5	4.2	0.6	1.3	1.5	3.5	0.3	3.8	0.5	1.6	0.4	1.5	0.3	0.8	1.6	0.5	3.5
0.4	11.3	0.1	0.3	1.1	0.1	1.1	0.3	2.5	1.5	4.7	0.3	4.2	0.7	1.2	0.9	2.6	0.7	6.5	0.3	*	0.4	0.5	0.0	1.0	1.9	0.7	3.6
0.6	6.5	0.0	0.2	5.9	0.1	1.1	0.3	2.2	1.2	4.3	0.7	4.0	0.5	1.1	0.9	2.3	0.1	5.0	0.2	0.4	0.2	0.8	0.2	0.5	1.6	0.2	2.9
0.6	7.4	0.0	0.4	10.7	0.1	0.8	0.5	2.8	1.8	5.5	1.4	2.8	0.5	1.1	1.4	3.6	0.1	6.2	0.3	1.6	0.2	1.2	0.1	0.6	1.2	0.6	3.5
1.3	10.6	0.0	0.4	4.4	0.3	1.5	1.0	4.0	3.5	6.4	3.5	4.9	0.9	1.6	2.2	4.0	0.4	4.8	0.7	1.8	0.6	1.6	0.5	0.6	1.4	1.3	4.4

CASEIN PEPTONES



The casein peptones are so named because of their starting material, milk. Milk is a very complex material, consisting of water, lactose, lipids, salts and proteins. It is the protein portion of the milk that provides the starting material for the casein peptones. After the milk has had the cream fraction removed and it is acidified (there are several methods available to accomplish this from the addition of acid to enzymatic and bacterial processes), the protein composition of

milk can be separated out.^{1,2} The soluble portion, known as whey protein, can be found in the supernatant. The insoluble portion, which precipitates out, consists of a group of heterogeneous phosphoproteins that exist together in a colloidal particle called a micelle. This group of phosphoproteins is collectively known as casein. The casein micelle can be broken down into four component classes, based in order of their decreasing mobility when separated by electrophoresis at pH 7, designated as α_{s1} , α_{s2} , β and κ casein. Casein, which can make up to 3% of the total components in bovine milk, is one of the most nutritive of the milk proteins, as it contains all of the common amino acids and is rich in the essential ones.

The casein recovered is known as acid casein and is insoluble in water. Generally, the acid casein is dissolved in a suitable hydroxide such as NaOH, to make it soluble in water. The resulting sodium caseinate is then used as the basis for hydrolyzed caseins. Sodium caseinate typically consists of between 87% to 90% protein.³ Hydrolyzed caseins are manufactured by one of two processes, acid hydrolysis or enzymatic hydrolysis.

Acid Hydrolysis

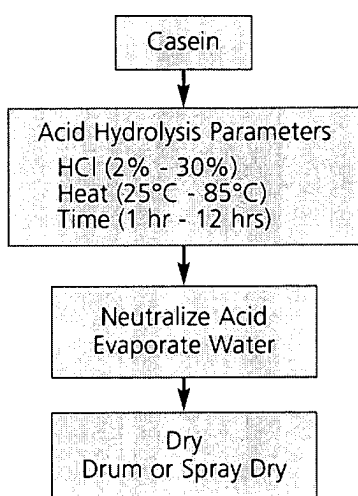
The diagram in Figure 1 shows the basic processing steps in the manufacture of a hydrolyzed casein by acid digestion. Many acids can be utilized but hydrochloric acid is most commonly used in the process.

This process leads to complete hydrolysis of the casein to amino acids and other compounds of relative chemical simplicity. However, not all of the amino acids survive intact. Tryptophan is typically destroyed in the process, as is cystine. Other amino acids, such as serine and threonine are reduced. Asparagine and glutamine, two amino acids with uncharged polar R groups, undergo hydrolysis to yield aspartic acid and glutamic acid, two amino acids which are negatively charged at pH 6 to 7.⁴

Enzymatic Hydrolysis

In animals, the stomach initiates the digestion of protein. However, it is the pancreas which carries out the majority of the protein breakdown. In the enzymatic hydrolysis of casein, the enzymes from the pancreas are utilized to manufacture these peptones. While the pancreas contains a battery of enzymes from the protease, lipase and amylase groups, it is the proteases, which are used in the hydrolysis of casein. The proteases found in the pancreas consist of trypsin, chymotrypsin, carboxypeptidase A, carboxypeptidase B and elastase. However, trypsin and chymotrypsin make up the greatest percentage of the total amount of proteases and carry out the bulk of the work. Both of these proteases have the ability to digest proteins into peptides, but they do not have the ability to break

Figure 1

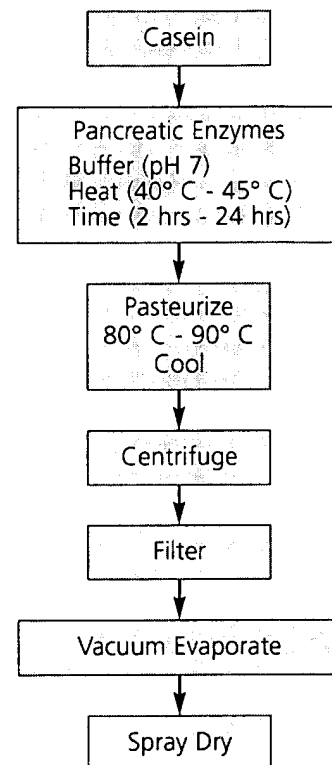


the protein down into its component amino acids. The carboxypeptidases do have this ability; however, they are not present in large enough amounts to accomplish this task to any great degree. Generally, in the digestion process of animals, these peptides would be broken down into amino acids through the actions of peptidases found in the epithelial cells of the small intestine.⁵ Thus, one of the characteristics of pancreatic digest of casein, as opposed to the acid hydrolysis of casein, is a peptone that consists of greater amounts of peptides. The basic processing steps involved in the manufacture of pancreatic digest of casein are shown in Figure 2.

References

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Figure 2



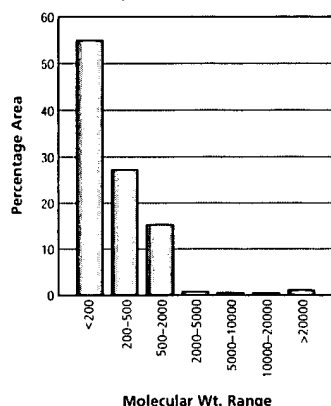
ACIDICASE™ PEPTONE

BACTO™ CASAMINO ACIDS

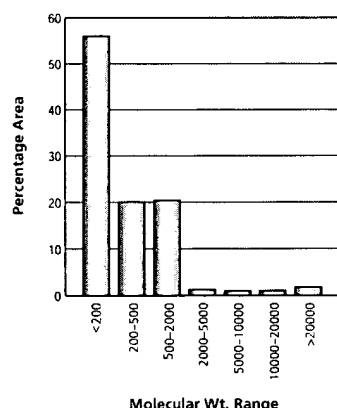
BACTO™ CASAMINO ACIDS, TECHNICAL

Molecular Weights

Acidicase Peptone



Bacto Casamino Acids



Acid Hydrolysates of Casein

Product Description

BBL™ Acidicase™ Peptone is a hydrochloric acid hydrolysate of casein. The manufacturing process produces a casein hydrolysate that has a high salt content of approximately 37% and nitrogen content of approximately 8%. The hydrolysis of the casein, a milk protein rich in amino acid nitrogen, is carried out until all the nitrogen is converted to amino acids or other compounds of relative simplicity. It is deficient in cystine, because casein contains little cystine, and in tryptophan, which is destroyed by the acid treatment.

Bacto™ Casamino Acids are an acid hydrolysate of casein, prepared according to the method described by Mueller and Miller.¹ The method described, reduces the sodium chloride and iron content of the hydrolyzed casein. This hydrolyzed casein, supplemented with inorganic salts, growth factors, cystine, maltose and an optimum amount of iron was used by Mueller and Miller to prepare diphtheria toxin. Bacto Casamino Acids duplicate this specially treated hydrolyzed casein.

Bacto Casamino Acids, Technical are prepared similarly to Bacto Casamino Acids but are less refined in the final processes.

Applications

BBL Acidicase Peptone is intended for use as a nutritional supplement in vitamin assay, susceptibility testing and other laboratory media and microbial fermentation where the high salt content will not interfere.

Bacto Casamino Acids, due to the nearly complete hydrolysis of casein and the low sodium chloride and iron content, make an excellent supplement for many media formulations where nitrogen requirements are minimal. It has been recommended as a compromise for the replacement of pure amino acids in a defined medium for the growth of *Lactobacillus*, thus eliminating the complexity of preparation.² Additionally, it has been successfully used, along with Tryptone Peptone in nutritional studies to determine a bacterium's growth requirement for peptides or amino acids.^{3,4} It also works well as a component in laboratory media. It has been utilized in such diverse applications as TYI-S-33 media for the parasite *Entamoeba histolytica* and LCM medium for the growth of a nematode-bacterium complex.⁵

Bacto Casamino Acids, Technical provides the same benefits as Bacto Casamino Acids, in instances where a less refined hydrolysate can be utilized.

Physical Characteristics

BBL Acidicase Peptone is a homogeneous, free-flowing, dehydrated powder; light beige in color.

Bacto Casamino Acids are a homogeneous, free-flowing, dehydrated powder; very light beige in color.

Bacto Casamino Acids, Technical are a homogeneous, free-flowing, dehydrated powder; very light beige in color.

Availability

BBL™ Acidicase™ Peptone 211843, 500 g

Bacto™ Casamino Acids 223050, 500 g

Bacto™ Casamino Acids 223020, 2 kg

Bacto™ Casamino Acids 223030, 10 kg

Bacto™ Casamino Acids, Technical, 223120, 500 g

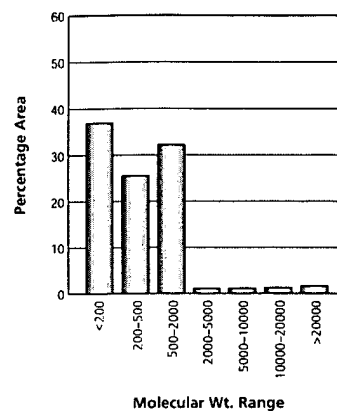
Bacto™ Casamino Acids, Technical, 223110, 10 kg

References

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5. Strauch and Ehlers. 2000. Influence of the aeration rate on the yields of the biocontrol nematode *Heterorhabditis megidis* in monoxenic liquid cultures. *Appl. Microbiol. Biotechnol.* **54**:9-13.

Molecular Weights

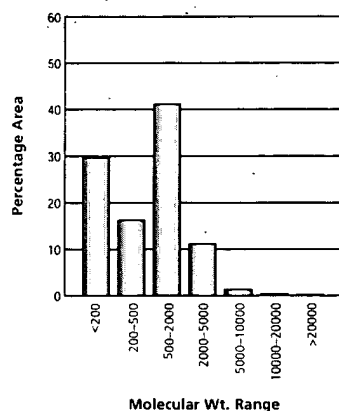
BiTek Casamino Acids



BIOSATE™ PEPTONE

Molecular Weights

Biosate Peptone



Product Description

Biosate™ Peptone is a mixed hydrolysate comprised of casein and yeast extract.

Applications

Biosate Peptone can be used as a component in microbiological media or in fermentation applications. The synergistic effect of two or more types of hydrolysates is well documented and has been utilized for decades in culture media formulation. The combination of pancreatic digest of casein and yeast extract provides nutritional benefits that are not provided by the components alone. It has been reported that the combined use of these two peptones has shown improved toxin production in clostridia.^{1,2} Additionally, the combination of pancreatic digest of casein and yeast extract has been used successfully as components in media which supported the hatching and culture of *Giardia* spp. from cysts and the first-time culturing of a nematode without the need of its symbiotic bacteria.^{3,4}

Physical Characteristics

Biosate Peptone is a homogeneous, free-flowing, dehydrated powder, yellow-tan in color.

Availability

Biosate™ Peptone 211862, 454 g

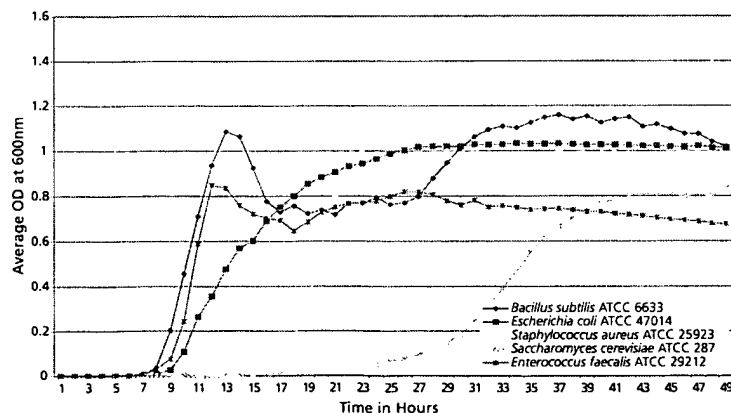
Biosate™ Peptone 294312, 25 lb (11.3 kg)

References

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3. Ponce, Martinez and Alvarez. 1989. Excystation and culture of *Giardia* spp. from human source. Archivos de Investigacion Medica 20:123-127.
4. Dorsman and Bijl. 1985. Cultivation of free-living stages of *Trichostrongylus colubriformis* in media without bacteria, animal tissue extract, or serum. J. Parasitol. 71:200-203.

Growth Curve

1% Biosate Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



BACTO™ CASITONE PEPTONE TRYPTICASE™ PEPTONE BACTO™ TRYPTONE PEPTONE BITEK™ TRYPTONE PEPTONE

Enzymatic Digests of Casein Product Description

Bacto™ Casitone Peptone is a pancreatic digest of casein. The manufacturing process for an enzymatic digest of casein is not as destructive as an acid hydrolysis. Thus, the casein is not broken down as completely into its constituent components. In many cases this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids.

Trypticase™ Peptone is a pancreatic digest of casein and is the primary nitrogen source in Trypticase Soy Broth and Agar.

Bacto Tryptone Peptone is a pancreatic digest of casein. It was developed by Difco Laboratories while investigating a peptone particularly suitable for the elaboration of indole by bacteria. It is also notable for the absence of detectable levels of carbohydrates.

BiTek™ Tryptone Peptone is prepared similarly to Bacto Tryptone Peptone but the final product goes through fewer refinement steps during processing.

Applications

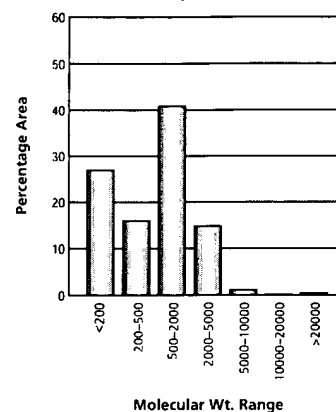
Bacto Casitone Peptone can be used as a component in microbiological media or in fermentation applications. A recent publication has also reported that the stability of lyophilized influenza virus vaccine was augmented by the addition of 2% Casitone.¹

Trypticase Peptone is recommended for use in media formulations, where good growth of fungi and bacteria is required. It is referenced in *Official Methods of Analysis of AOAC International* and meets the USP specifications for pancreatic digest of casein.^{2,3}

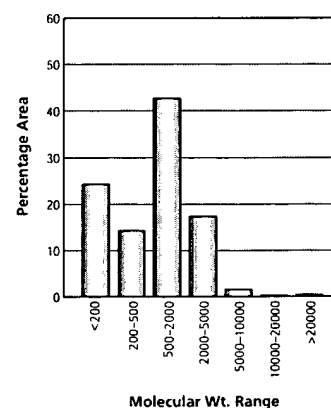
Bacto Tryptone Peptone has been used in conjunction with Casamino Acids in nutritional studies to determine amino acids vs. peptide utilization.^{4,5} It is included in standard

Molecular Weights

Bacto Casitone Peptone

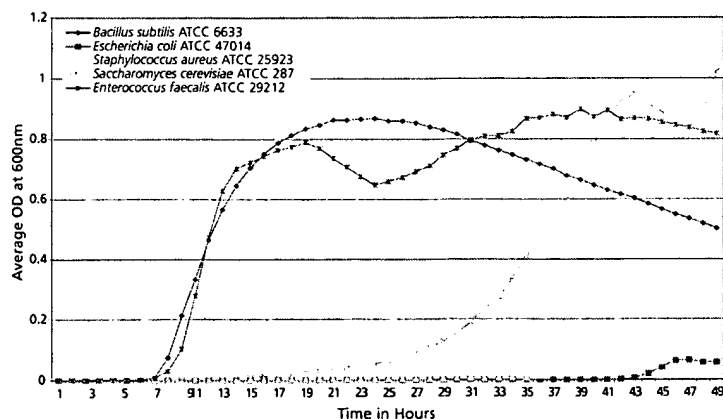


Trypticase Peptone



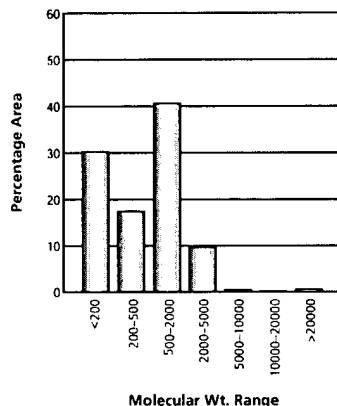
Growth Curve

1% Bacto Casitone Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C

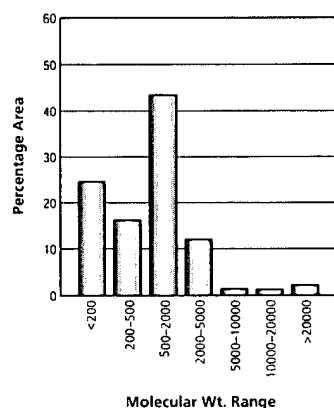


Molecular Weights

Bacto Tryptone Peptone



BiTek Tryptone Peptone



methods manuals applications and is listed in the "Reagent" section of *The United States Pharmacopeia*, as meeting the specifications for pancreatic digest of casein, a component in many of the media listed.^{2,3,6-9} *The European Pharmacopoeia* also lists pancreatic digest of casein as a component in many of the recommended media.¹⁰ Bacto™ Tryptone Peptone also works well in fermentation applications. It has been used successfully with commonly used organisms such as *Escherichia coli*,¹¹ as well as uncommon organisms such as the diatom *Nitzschia laevis*.¹²

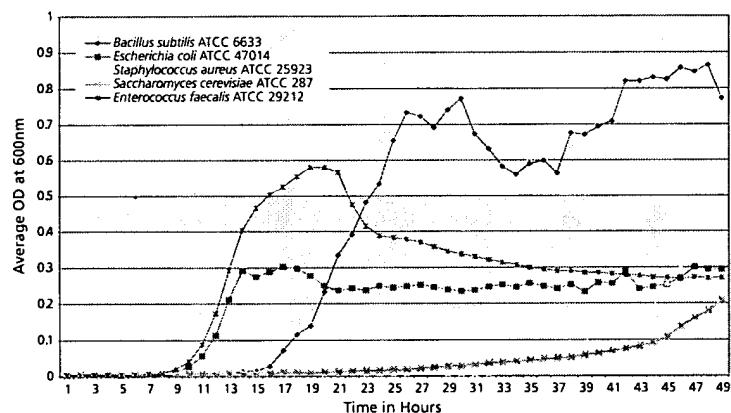
BiTek™ Tryptone Peptone provides some of the same benefits as Bacto Tryptone in instances where a less refined hydrolysate can be utilized.

Physical Characteristics

Bacto Casitone Peptone is a homogeneous, free-flowing, dehydrated powder, tan in color.

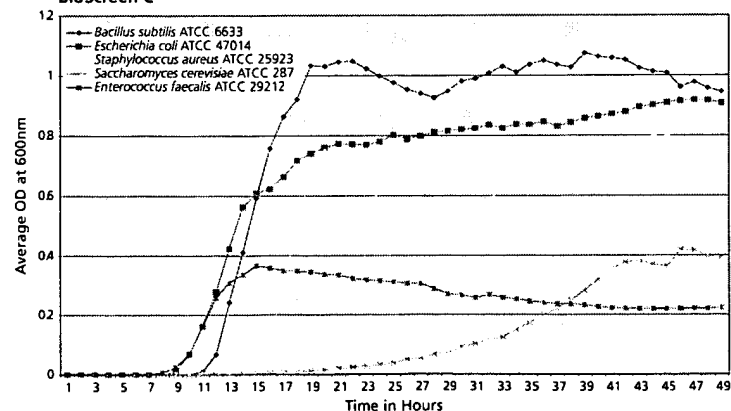
Growth Curve

1% Trypticase Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Growth Curve

1% Bacto Tryptone Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



BBL Trypticase Peptone is a homogeneous, free-flowing, dehydrated powder; very light beige in color.

Bacto Tryptone Peptone is a homogeneous, free-flowing, dehydrated powder; light beige in color

BiTek Tryptone Peptone is a homogeneous, free-flowing, dehydrated powder; beige in color.

Availability

Bacto™ Casitone Peptone 225930, 500 g

Bacto™ Casitone Peptone 225910, 10 kg

BBL™ Trypticase™ Peptone 211921, 454 g

BBL™ Trypticase™ Peptone 211922, 5 lb (2.3 kg)

BBL™ Trypticase™ Peptone 211923, 25 lb (11.3 kg)

Bacto™ Tryptone Peptone 211705, 500 g

Bacto™ Tryptone Peptone 211699, 2 kg

Bacto™ Tryptone Peptone 211701, 10 kg

BiTek™ Tryptone Peptone 251420, 10 kg

References

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CASEIN PEPTONES TYPICAL ANALYSES

Product Name	Total Nitrogen (%)	Amino Nitrogen (%)	AN/TN	Total Carbohydrate (mg/g)	Ash (%)	Loss on Drying (%)	NaCl (%)	pH (2% Solution)	Calcium (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% Free)	Alanine (% Total)	Arginine (% Free)	Arginine (% Total)	Asparagine (% Free)	Aspartic Acid (% Free)	Aspartic Acid (% Total)	Cystine (% Free)
Acidicase™ Peptone	8.5	6.2	0.73	0.29	36.8	5.3	32.3	6.8	229	36	383	140900	16.99	0.25	1.42	1.6	2.1	1.3	1.9	0.0	3.4	3.9	0.8
Biosate™ Peptone	13.4	6.0	0.45	32.98	7.7	6.6	0.3	7.1	258	398	21320	17100	0.07	0.43	3.19	2.4	4.2	2.1	2.9	0.9	0.9	5.9	0.3
Casamino Acids, Bacto™	10.8	9.4	0.87	0.0	18.3	4.8	12.1	6.4	59	143	4098	88090	6.74	0.55	2.56	3.0	3.0	2.4	2.5	0.0	5.0	5.0	0.1
Casamino Acids, Technical, Bacto	8.3	5.9	0.71	0.15	36.0	1.2	30.1	6.9	110	48	1361	145667	18.25	0.26	1.53	2.1	4.4	1.1	1.7	0.0	3.6	3.2	0.4
Casitone, Bacto	13.5	5.0	0.37	3.54	6.4	2.0	0.0	7.0	111	213	3480	34090	0.10	0.40	2.48	0.9	3.4	2.6	2.8	0.5	0.2	5.5	0.0
TC Lactalbumin Hydrolysate	13.0	6.3	0.48	21.01	7.2	4.6	0.3	7.0	1620	340	17200	14800	0.80	1.20	4.10	2.3	4.7	2.2	2.5	0.9	0.9	6.5	0.2
Trypticase™ Peptone	14.2	5.2	0.37	3.99	5.7	4.0	0.1	7.2	295	110	588	26600	0.09	0.18	2.54	0.9	5.7	2.3	4.8	0.5	0.2	7.7	0.3
Tryptone, Bacto	13.3	5.3	0.40	4.30	6.6	2.3	0.0	7.3	256	195	3257	33910	0.06	0.33	2.58	1.0	3.2	3.1	2.7	0.6	0.4	5.2	0.3
Tryptone, BiTek™	13.1	5.6	0.43	8.42	5.8	5.0	0.0	7.1	387	100	620	26970	0.35	0.22	2.25	0.6	5.0	3.8	2.6	0.5	0.1	3.9	0.4

* = Partially destroyed during hydrolysis

0.0 = Below limits of detection

For test methods see Definition of Methods section on page 61.

Free Amino Acids / Total Amino Acids

Glutamic Acid (% Free)	Glutamic Acid (% Total)	Glutamine (% Free)	Glycine (% Free)	Glycine (% Total)	Histidine (% Free)	Histidine (% Total)	Isoleucine (% Free)	Isoleucine (% Total)	Leucine (% Free)	Leucine (% Total)	Lysine (% Free)	Lysine (% Total)	Methionine (% Free)*	Methionine (% Total)	Phenylalanine (% Free)	Phenylalanine (% Total)	Proline (% Free)	Proline (% Total)	Serine (% Free)	Serine (% Total)*	Threonine (% Free)	Threonine (% Total)	Tryptophan (% Free)	Tyrosine (% Free)	Tyrosine (% Total)	Valine (% Free)	Valine (% Total)
8.3	11.6	0.0	0.8	1.0	0.8	1.6	1.6	4.0	3.9	6.3	4.4	4.6	0.9	1.4	2.5	3.5	3.3	5.3	2.2	1.8	0.9	1.4	0.0	1.0	1.4	1.8	4.4
3.5	16.1	0.3	0.6	2.2	0.6	2.0	1.6	5.8	4.7	7.7	3.5	5.9	1.0	1.9	2.9	5.5	0.5	6.2	1.0	2.2	0.8	1.9	0.7	0.5	1.4	1.9	6.1
15.1	15.9	0.0	1.4	1.4	2.0	1.9	3.1	4.0	4.6	5.0	6.0	5.9	1.4	1.4	3.4	3.6	7.5	8.0	4.3	2.0	2.0	1.7	0.0	0.4	0.4	4.7	5.6
5.1	8.4	0.0	0.8	1.1	0.5	1.1	1.2	2.7	2.7	4.6	4.0	4.6	0.9	1.2	1.4	1.9	2.9	5.7	1.8	0.2	0.9	0.5	0.0	1.5	1.6	1.6	3.4
0.9	16.0	0.0	0.2	1.7	0.4	1.9	1.1	5.9	4.7	7.9	4.5	5.9	1.1	2.2	2.7	5.5	0.3	7.1	0.8	2.1	0.5	1.9	0.8	0.5	1.6	1.3	6.3
6.6	8.7	0.3	1.3	2.7	0.5	1.1	2.1	3.6	3.5	4.9	4.9	2.3	4.2	0.8	0.8	2.3	3.3	0.9	1.5	1.4	1.3	1.4	0.6	0.8	0.9	2.4	3.7
1.1	13.2	0.1	0.1	6.3	0.5	4.8	1.1	8.3	5.3	10.4	3.3	10.6	1.1	2.5	2.7	7.1	0.2	10.9	0.4	2.5	0.6	2.4	0.8	0.4	1.6	1.5	9.1
1.4	15.1	0.1	0.2	1.7	0.5	1.9	1.3	5.5	4.8	7.5	5.5	6.2	1.0	2.1	3.0	5.2	0.2	6.6	0.7	2.2	0.7	1.8	0.8	0.5	1.3	1.7	5.9
0.7	9.8	0.1	0.1	1.4	0.6	1.6	1.1	3.8	4.2	6.0	5.4	5.9	0.7	1.4	2.8	3.4	0.1	7.3	0.7	0.3	0.7	0.8	0.8	0.4	1.2	1.5	4.6

ANIMAL-FREE PEPTONES, YEAST EXTRACTS AND MEDIA



The need for animal-free products has increased due to rising concerns about BSE/TSE. In response to this need, BD has developed a broad offering of soy peptones, yeast extracts and animal-free media.

In addition to the more common animal-free components such as soy and yeast, many new plant-based products are being tested on the market. Wheat, pea and potato have become candidates of experimental interest. These plant-based materials offer a new supplementation source with potentially strong performance characteristics.

Soy Peptone, one of the first successful plant-based peptones to be optimized into cell culture, are processed in several different ways to provide various nutrient mixes. Yeast Extracts and Tissue Culture (TC) Yeastolates offer a different range of nutritional choices to enhance production in both bacterial fermentation and (as a supplement) in cell culture.

Soy Peptones

The BD Difco™ Soy Peptones are all enzymatic digests of soy flour. Soy contains several heat labile protease inhibitors.¹ The most common way of eliminating these factors is to heat or toast the defatted soy beans in a processing plant under controlled conditions. Soy flour, the principle substrate in a soy peptone, is rich in high-quality protein, carbohydrates, calcium and B vitamins.² The enzymes used in the digestion of soy flour should be from animal-free sources or from microorganisms that have been grown in animal-free media.

Yeast Products

Yeast extract is defined in the USP as "a water-soluble, peptone-like derivative of yeast cells (*Saccharomyces*)."³ Yeast extract is readily available in the U.S. as a spray-dried powder. In Europe, pharmaceutical companies use it as a liquid or paste, as well as in the powdered form.

Yeast extract is used by the health food industry as an inexpensive source of vitamins, and has long been recognized as a major source of B-complex vitamins. Yeast extract, as a substrate in a media formulation, supplies not only vitamins, but also proteins, carbohydrates and some micronutrients.

There are many kinds of yeast extract. The two principle sources of yeast extract are "brewer's" yeast and "baker's" yeast. Brewer's yeast is a by-product from the brewing industry. It requires de-bittering (removal of hop resins) before it is suitable for fermentation use.⁴ A wide variety of strains and growth processes have been used in the manufacture of brewer's yeast, thus precluding any consistency of the final product.

Baker's yeast (*Saccharomyces cerevisiae*) is defined as a primary yeast because the yeast is grown for the specific purpose of being used as a substrate in a bioprocess or as a food product/flavoring. Manufacture of baker's yeast is a reproducible and controlled process. The yeast organism is grown on a molasses-based medium optimized for the specific



yeast.⁵ Commercial yeast fermentations are always fed-batch type fermentations lasting from 12-20 hours.⁶ Commercial baker's yeast manufacturers have found that the more highly aerated a culture, the higher the final product yield.⁶

The process of manufacturing baker's yeast extract is unique compared to the manufacture of peptones. Yeast extract is an autolysate; i.e. cell hydrolysis is performed by the endogenous enzymes of the *Saccharomyces* organism. Autolysis is usually begun by either a controlled temperature shock or, for the food industry, an osmotic shock, which causes the yeast cells to expire. The temperature shock is not high enough to inactivate the proteases of the yeast cell, which proceed to degrade the cell. Autolysis can proceed from 10 to 60 hours. After autolysis, soluble material is separated from the insoluble material by means of centrifugation and several filtration steps.⁶ The final filtration product is concentrated and then spray dried, or can be left in the concentrated paste form, which contains approximately 60-80% solids.

Temperature, pH, addition of other enzymes, type of medium substrate for the growth of the *Saccharomyces* and duration of autolysis are all variables that create the large variety of yeast extracts available.

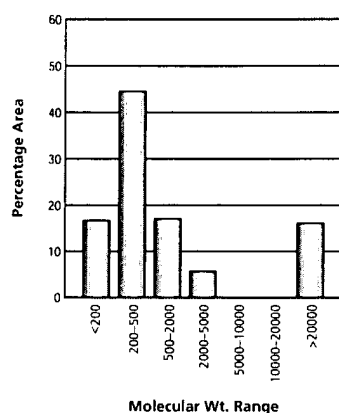
References

1. Kunitz, M. 1945. Crystallization of a trypsin inhibitor from soybeans. *Science* 101, p.668-669.
2. Human Nutrition Service, U.S. Department of Agriculture. 1986. Composition of foods: legume and legume products. Agriculture handbook, No. 8-16, revised. U.S. Department of Agriculture, Washington, D.C.
3. United States Pharmacopeial Convention, Inc. 2004. The United States pharmacopeia 27/The national formulary 22-2004. United States Pharmacopeial Convention, Inc., Rockville, Md.
4. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Methods in microbiology, volume 3A, Academic Press, p.252-56.
5. <http://www.ohly.ed/sommer.htm>.
6. Reed and Nagodawithana. Yeast technology, 2nd ed., p.264-267. Van Nostrand Reinhold, New York.

BACTO™ MALT EXTRACT

Molecular Weights

Bacto Malt Extract



Product Description

Bacto™ Malt Extract is the water-soluble portion of malted barley. The extraction process breaks down the polysaccharides into simple sugars. After the malting process is complete the extract is prepared from the malted barley by cracking the grain in a mill and then extracting the grain with a warm liquor. The resulting “wort” is filtered and evaporated or dried under vacuum.^{1,2}

Applications

Bacto Malt Extract is used in the culture of yeasts and molds. Bacto Malt Extract is very high in carbohydrate content.³ This product is suitable for the culture of yeasts and molds because of the high concentration of reduced sugars, especially the maltoses. Malt extract in the agar form is recommended for the detection and isolation of yeasts and molds from dairy products and foods and as a medium for stock culture maintenance.

Physical Characteristics

Bacto Malt Extract is a medium tan, free-flowing, homogeneous powder.

Availability

Bacto™ Malt Extract 218630, 500 g

Bacto™ Malt Extract 218610, 10 kg

References

1. Bridson and Brecker. 1970. In Norris and Ribbons (ed.), *Methods in microbiology*, vol. 3A, p. 256. Academic Press, New York.
2. How malt is made, Briess Malting Company. 2 Dec. 2002. <<http://www.briess.com/HomebrewNew/hbhow.htm>>
3. Cote. 1999. In Flickinger and Drew (ed.), *Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation*, p. 1652. John Wiley & Sons, Inc., New York.

DIFCO™ SPRINGER™ DS100 SOY PEPTONE UF PHYTONE™ PEPTONE PHYTONE™ PEPTONE UF DIFCO™ SELECT SOY TONE BACTO™ SOY TONE

Product Description

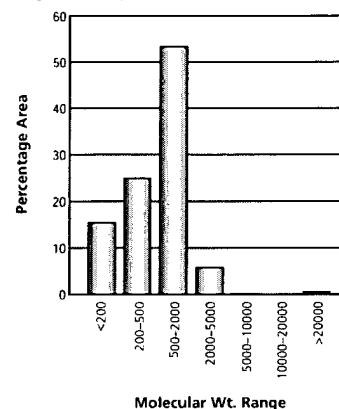
All of the Difco™ and BBL™ brand soy peptones are enzymatic digests of soybean meal/flour. They are recommended for use in media for the cultivation of a wide variety of organisms, including fungi. The soybean protein in these peptones contains naturally occurring high concentrations of vitamins and carbohydrates.

Applications

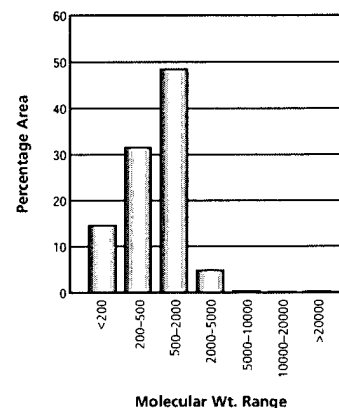
BD offers a diverse choice of soy peptones. The individual characteristics of each peptone are the result of processing methods engineered to consistently deliver these characteristics from batch to batch. The nutritional requirements of microorganisms and cell lines vary according to each individual strain. While some organisms or cell lines may prefer

Molecular Weights

Phytone Peptone

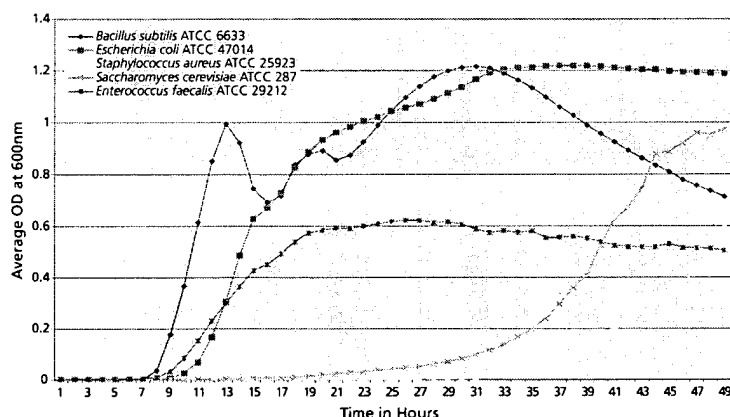


Phytone Peptone UF



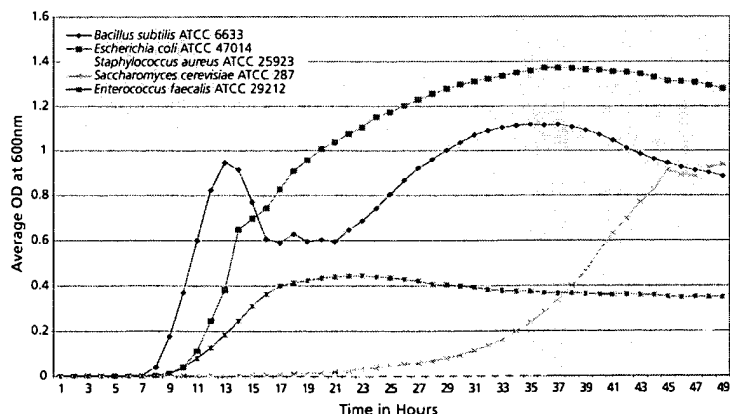
Growth Curve

1% Phytone Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



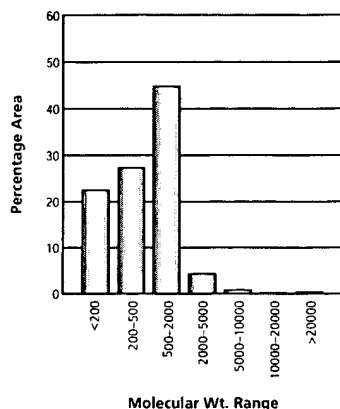
Growth Curve

1% Phytone Peptone UF in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C

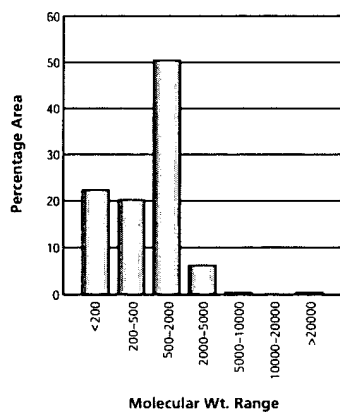


Molecular Weights

Select Soytone



Bacto Soytone



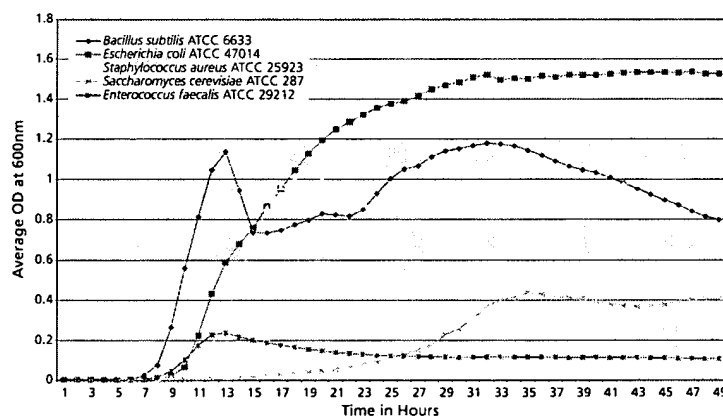
short chain or free amino acids, others benefit from longer chain amino acids. While the typical analysis profiles for each peptone in this manual can help direct the end-user to the correct peptone match, it is recommended that end-users supplement the typical analytical information with evaluations in their own individual growth models.

BD offers two ultrafiltered soy peptone products suitable for cell culture purposes;

Difco™ Springer™ DS100 Soy Peptone UF is an animal-free product processed in dedicated animal-free equipment. This product is intended for use as a nutritional supplement in cell culture, laboratory media and microbial fermentation. Low endotoxin levels make Difco Springer DS100 Soy Peptone UF an ideal substitute for serum. It may also be used in highly concentrated feed solutions for bacterial fermentation applications. Cell viability results yielded greater than or equal to 85% viability with Mink Lung cells for murine and feline sarcoma virus assay (ML ATCC™ CCL-64) and with Green Monkey Kidney cells for verotoxin detection (VERO ATCC CCL-81). DS100 Soy Peptone UF has an endotoxin level of less than 300 EU/g.

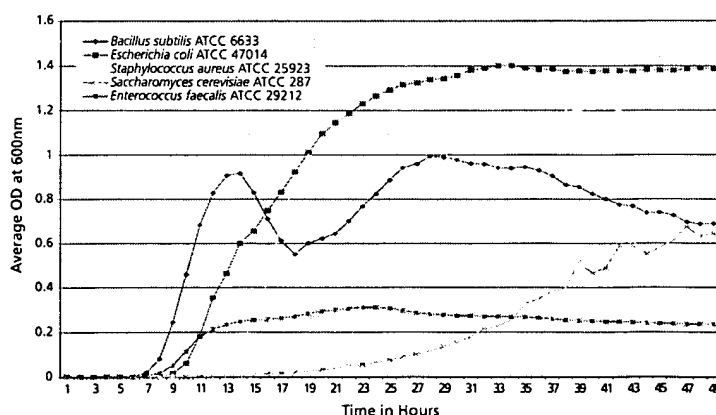
Growth Curve

1% Select Soytone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Growth Curve

1% Bacto Soytone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Phytone™ Peptone UF is an ultrafiltered peptone that was developed specifically for the tissue culture market. Its nitrogen content combined with the naturally occurring vitamins has demonstrated remarkable growth support with monoclonal antibodies and protein expression. It has an endotoxin level of less than or equal to 500 EU/g.

BD offers three other soy peptone products suitable for a variety of bacterial cultures.

Phytone Peptone is an animal-free soy peptone. Phytone Peptone retains the high vitamin and high carbohydrate content of the soy plant tissue. It is an excellent plant peptone for the cultivation of fungi and fastidious types of bacteria, such as members of the *Clostridium* and *Neisseria* genera.¹ It has been used in cell culture applications due to its high carbohydrate content.

Select™ Soytone demonstrates excellent growth support for *Escherichia coli*. Select Soytone is also used in Select APS™ Super Broth. Subtle differences in the digestion process give Select Soytone improved performance in cell culture (see page 8).

Bacto™ Soytone was found to be effective in the recovery of stressed *E. coli*.² It was found that Bacto Soytone with the addition of 7 vitamins replaced yeast extract as an economical alternative for the production of lactic acid by *Lactobacillus rhamnosus*.³ It should be noted that Bacto Soytone utilizes an animal based enzyme in the digestion of the soy flour.

Physical Characteristics

Difco Springer DS100 Soy Peptone UF is a medium tan, free-flowing, homogenous powder.

BBL Phytone Peptone is a light tan, free-flowing, homogeneous powder.

BBL Phytone Peptone UF is a light tan, free-flowing, homogeneous powder.

Difco Select Soytone is a tan, free-flowing, homogeneous powder.

Bacto Soytone is a light to medium tan, free-flowing, homogeneous powder.

Availability

Difco™ Springer™ DS100 Soy Peptone UF 220515, 500 g

Difco™ Springer™ DS100 Soy Peptone UF 220516, 10 kg

BBL™ Phytone™ Peptone 211906, 454 g

BBL™ Phytone™ Peptone 298147, 5 lb (2.3 kg)

BBL™ Phytone™ Peptone 292450, 10 kg

BBL™ Phytone™ Peptone UF 210931, 500 g

BBL™ Phytone™ Peptone UF 210936, 10 kg

Difco™ Select™ Soytone 212488, 500 g

Difco™ Select™ Soytone 212489, 10 kg

Bacto™ Soytone 243620, 500 g

Bacto™ Soytone 243610, 10 kg

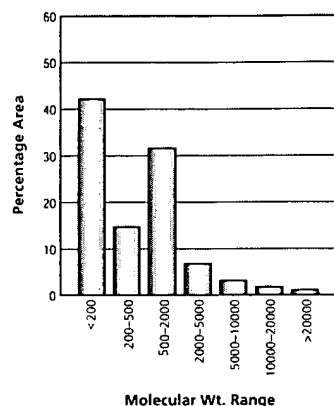
References

1. Power (ed.), 1988. Manual of BBL™ products and laboratory procedures, 6th ed., p.293. Becton Dickinson Microbiology Systems, Cockeysville, Md.
2. Chou and Cheng. 2000. Recovery of low-temperature stressed *E. coli* O157:H7 and its susceptibility to crystal violet, bile salt, sodium chloride and ethanol. Int. J. Food Microbiol. 61:127-136.
3. Kwon, Lee, Lee, Chang, Keun and Chang. 2000. Production of lactic acid by *Lactobacillus rhamnosus* with vitamin-supplemented soybean hydrolysate. Enzyme Microb. Technol. 26:209-215.

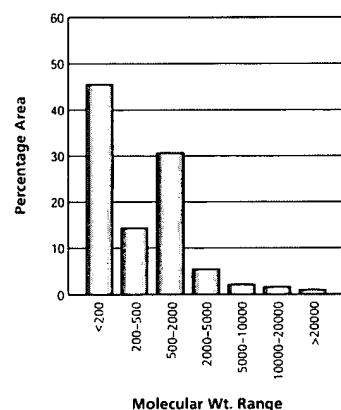
BACTO™ TC YEASTOLATE TC YEASTOLATE, UF

Molecular Weights

Bacto TC Yeastolate



TC Yeastolate, UF



Product Description

TC Yeastolate products are animal-free and water-soluble portions of autolyzed yeast or *Saccharomyces cerevisiae*. TC Yeastolate is a mixture of peptides, amino acids, carbohydrates, simple and complex as well as vitamins. TC Yeastolate, UF has been ultrafiltered at a 10,000 MWCO (Molecular Weight Cut-Off). It has an endotoxin value of less than 500 EU/g.

Applications

TC Yeastolate products are intended as nutritional supplements for bacterial, insect and mammalian cell culture. TC Yeastolate has been used in insect cell nutrition. TC Yeastolate was found to be a very versatile supplement to enhance growth and production characteristics of Sf9 and High-Five cells.¹⁻⁵

Physical Characteristics

Bacto™ TC Yeastolate is a beige free-flowing, homogeneous, spray-dried powder.

TC Yeastolate, UF is a free-flowing, homogeneous, spray-dried powder.

Availability

Bacto™ TC Yeastolate 255772, 100 g

Bacto™ TC Yeastolate 255771, 10 kg

Bacto™ TC Yeastolate 292731, 25 kg

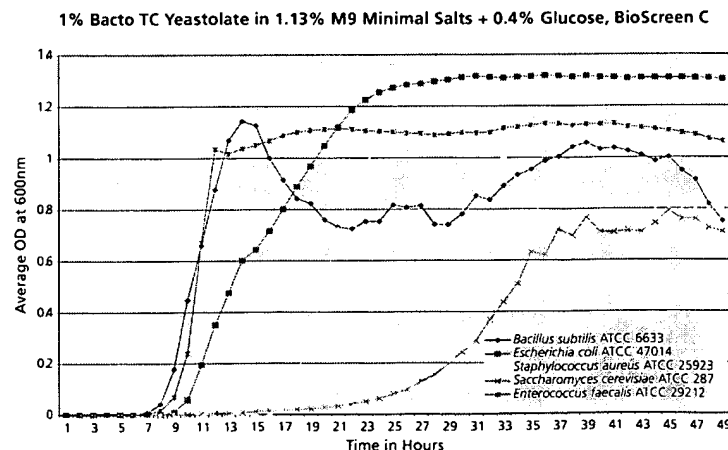
TC Yeastolate, UF 292804, 500 g

TC Yeastolate, UF 292805, 10 kg

References

1. Chan, Greenfield and Reid. 1998. Optimising fed-batch production of recombinant proteins using the baculovirus expression vector system. *Biotechnol. Bioeng.* 59:178-188.
2. Nguyen, Jarnagin, Williams, Chan and Barnett. 1993. Fed-batch culture of insect cells: a method to increase the yield of recombinant human nerve growth factor (rhNGF) in the baculovirus expression system. *J Biotechnol.* 31:205-217.
3. Ikonou, Bastin, Schneider, Agathos. 2001. Design of efficient medium for insect cell growth and recombinant protein production. *In Vitro Cell Dev. Biol. Anim.* 37:549-559.
4. Bedard, Kamen, Tom and Maassie. 1994. Maximization of recombinant protein yield in the insect cell/baculovirus system by one-time addition of nutrients to high-density batch cultures. *Cytotechnology* 15:129-138.
5. Donaldson and Shuler. 1998. Low-cost serum-free medium for the BTI-TN5B1-4 insect cell line. *Biotechnology Prog.* 14:573-579.

Growth Curve



BACTO™ YEAST EXTRACT

YEAST EXTRACT

YEAST EXTRACT, LD

YEAST EXTRACT, UF

Product Description

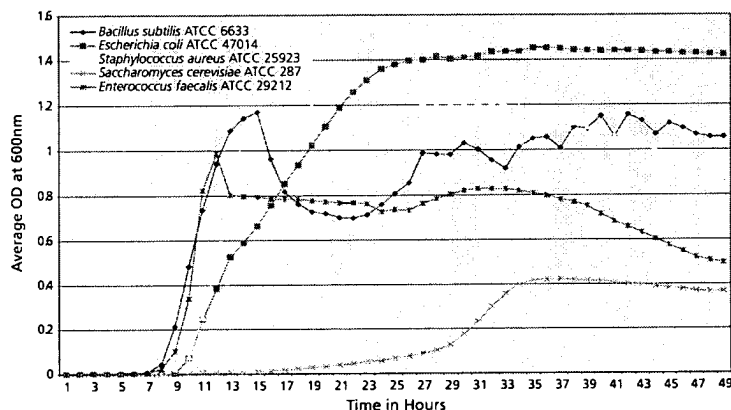
BD Yeast Extracts are concentrates of the water soluble portion of *Saccharomyces cerevisiae* cells that have been autolyzed. BD Yeast Extracts are derived from primary grown baker's yeast. Yeast extract is an animal-free product and is used extensively for many animal-free formulations for bacterial, fungal, mammalian and insect cell culture. Yeast Extract will provide essential water soluble vitamins, amino acids, peptides and carbohydrates to any medium formulation

Applications

BD Yeast Extracts are animal-free products suitable for use as multi-functional nutritional supplements in cell culture, microbial fermentation and insect cell culture applications.

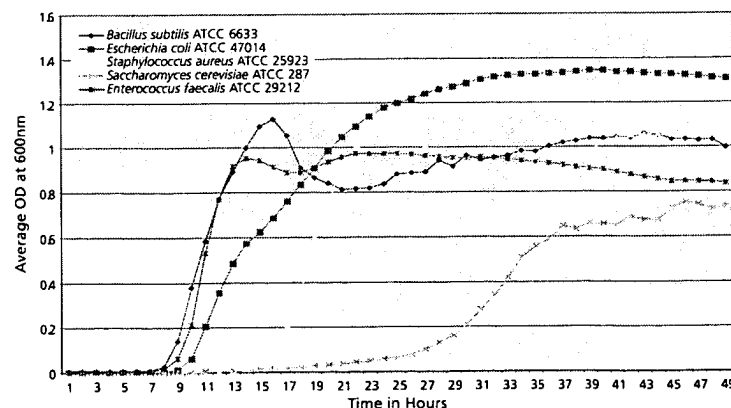
Growth Curve

1% Bacto Yeast Extract in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



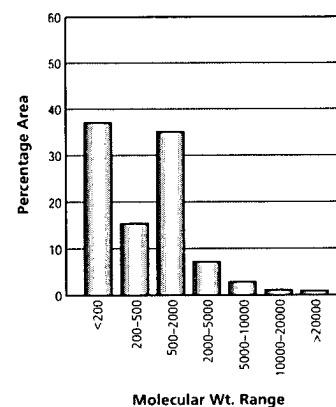
Growth Curve

1% Yeast Extract 211929 in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C

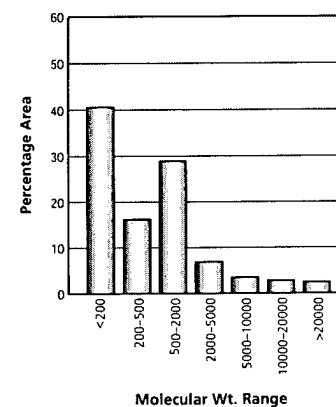


Molecular Weights

Bacto Yeast Extract

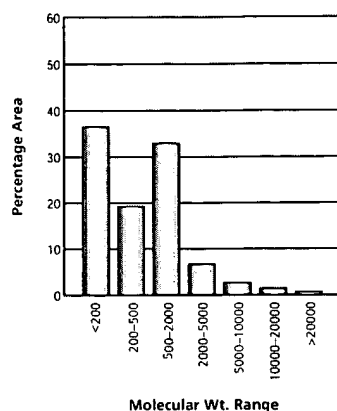


Yeast Extract

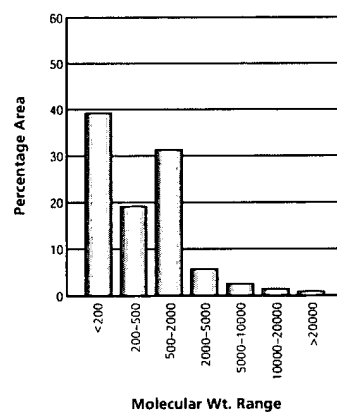


Molecular Weights

Yeast Extract, UF



Yeast Extract, LD



Bacto™ Yeast Extract is one of the most complete and versatile fermentation bionutrients available. It is an important ingredient for the microbiological assay of vitamins. Yeast extract is also of value in the assay of antibiotics. B factor, a growth substance necessary for the production of rifampin in a *Nocardia* sp., can be isolated from yeast extract.¹

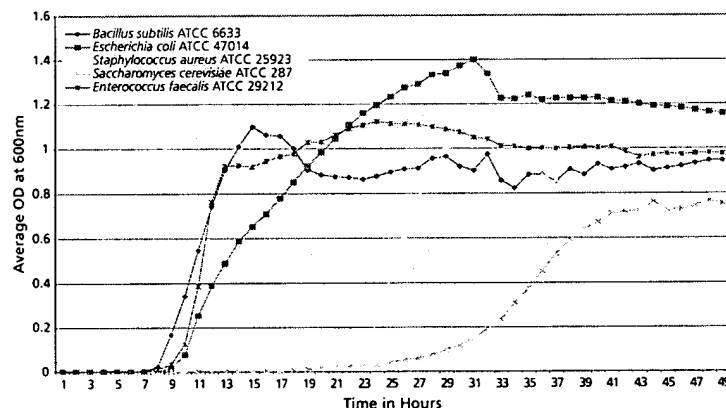
Yeast Extract, UF is ultrafiltered and specifically designed for tissue culture applications. With its low endotoxin level and high content of naturally occurring B vitamins, it is an ideal substitute for fetal bovine serum. It has an endotoxin level of less than or equal to 500 EU/g.

Yeast Extract, LD was developed to reduce the problem of dust inhalation when handling large quantities of yeast extract. Yeast Extract, Yeast Extract UF, and Yeast Extract LD are processed from the same strain of *Saccharomyces*.

Yeast Extract was developed to provide a product for the biotechnology/pharmaceutical market with acceptable clarity and growth promoting characteristics.

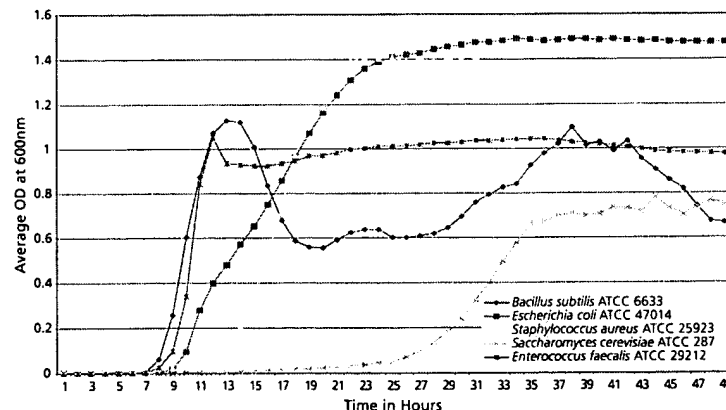
Growth Curve

1% Yeast Extract UF in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Growth Curve

1% Yeast Extract LD in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Media formulations containing yeast extract are specified in standard methods for various applications.²⁻⁸

Physical Characteristics

BD Yeast Extracts are beige free-flowing, homogeneous, spray-dried powders.

Availability

Bacto™ Yeast Extract 212750, 500 g

Bacto™ Yeast Extract 212720, 2 kg

Bacto™ Yeast Extract 212730, 10 kg

Bacto™ Yeast Extract 212710, 50 kg

Yeast Extract 211929, 454 g

Yeast Extract 211930, 5 lb (2.3 kg)

Yeast Extract 211931, 25 lb (11.3 kg)

Yeast Extract, UF 210929, 500 g

Yeast Extract, UF 210934, 10 kg

Yeast Extract, LD 210933, 500 g

Yeast Extract, LD 210941, 10 kg

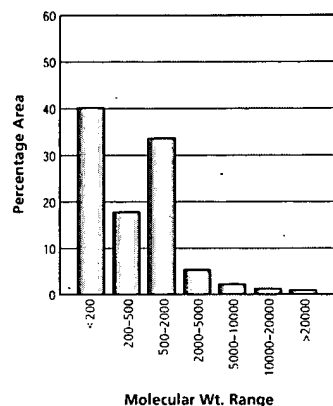
References

1. Kawauchi, Asahi, Satoh, Uozumi and Beppu. 1984. J. Antibiot. 37:1587.
2. Horowitz (ed.) 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.
3. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, Md.
4. Downes and Ito (ed.) 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. U.S. Environmental Protection Agency (USEPA). 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington D.C.
6. Marshall (ed.) . 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
7. Clesceri, Greeberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
8. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food Safety and Inspection Service, USDA , Washington, D.C.

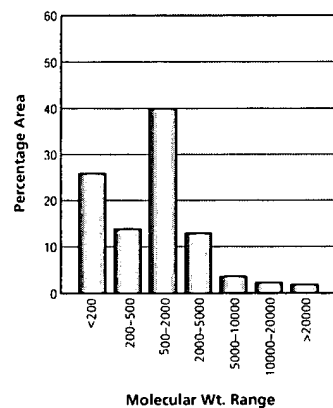
SELECT APS™ SUPER BROTH SELECT APS™ LB BROTH

Molecular Weights

Select APS Super Broth



Select APS LB Broth



Product Description

The Select APS™ media line is manufactured from animal-free ingredients. These Alternative Protein Source (APS) media are nutrient-rich formulations designed to out-perform classical meat-based formulations (see growth curves below).

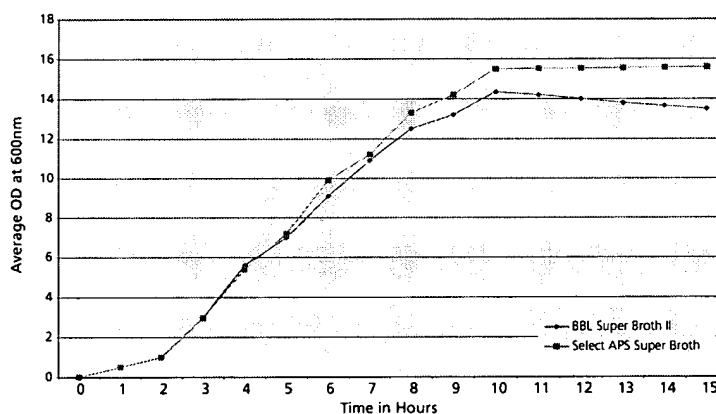
Applications

Select APS Super Broth is a molecular genetic medium designed to grow *Escherichia coli* to a high cell density. There is no glucose in the formulation thus preventing acetate build-up in the fermentation of the organism.¹

Select APS LB Broth is an excellent all-purpose growth medium for the propagation and maintenance of *E. coli* in molecular biology procedures.

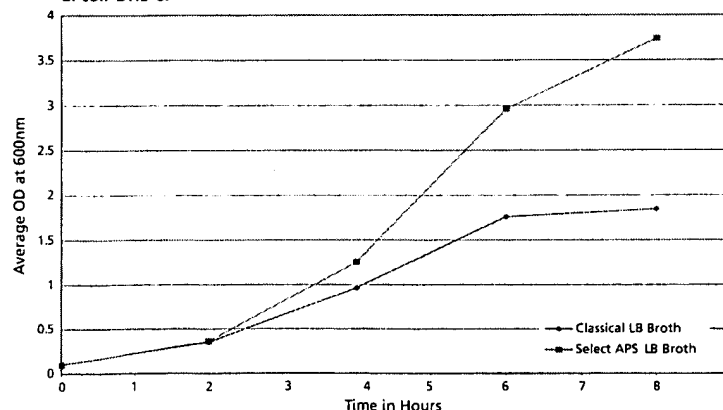
Growth Curve

Growth Performance of BBL Super Broth II vs. Select APS Super Broth *E. coli* DH5α



Growth Curve

Growth Performance of Classical LB Broth Base vs. Select APS LB Broth *E. coli* DH5 α



Physical Characteristics

Select APS Super Broth is a tan, free-flowing powder.

Select APS LB Broth is a tan, free-flowing powder.

Availability

Select APS™ Super Broth 212485, 500 g

Select APS™ Super Broth 212486, 10 kg

Select APS™ LB Broth 292438, 500 g

Select APS™ LB Broth 212484, 10 kg

Reference

1. Swartz. 2001. Advances in *Escherichia coli* production of therapeutic proteins. *Curr. Opin Biotechnology* 12:195-201.

ANIMAL-FREE PEPTONE AND YEAST EXTRACTS

TYPICAL ANALYSES

Product Name	Total Nitrogen (%)	Amino Nitrogen (%)	AN/TN	Total Carbohydrate (mg/g)	Ash (%)	Loss on Drying (%)	NaCl (%)	pH (2% Solution)	Calcium (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% Free)	Alanine (% Total)	Arginine (% Free)	Arginine (% Total)	Asparagine (% Free)	Aspartic Acid (% Free)	Aspartic Acid (% Total)	Cystine (% Free)
DS100 Soy Peptone UF	10.6	4.2	0.4	116.7	12.2	3.0	0.5	6.9	2057	4385	74737	921	0.57	0.34	1.71	1.4	3.1	0.7	4.4	0.7	1.1	7.2	0.6
Malt Extract, Bacto™	0.3	0.3	0.97	1037.4	0.3	3.1	0.2	5.2	111	130	603	713	0.07	0.07	0.08	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.0
Phytone™ Peptone	9.0	2.4	0.27	392.9	12.4	1.5	4.0	7.1	1001	2435	31547	34037	0.76	0.67	0.64	0.3	2.6	0.6	2.1	0.1	0.3	3.9	0.4
Phytone Peptone UF	9.4	2.6	0.28	394.2	12.5	4.9	4.0	7.0	900	1700	21200	36100	0.76	0.58	0.71	0.3	3.1	0.8	2.4	0.2	0.2	4.7	0.5
Select APS™ LB Broth	8.7	4.6	0.53	76.2	30.2	3.3	14.2	6.8	245	561	43880	88090	19.0	0.7	1.04	3.4	5.9	1.2	2.2	1.1	1.3	3.7	0.2
Select APS Super Broth	8.1	6.2	0.78	79.4	31.8	2.4	1.1	7.3	155	1066	138700	8212	0.1	0.9	2.62	2.1	2.9	1	1.8	0.9	0.9	4.1	0.2
Select Soytone	9.2	3.7	0.40	336.2	10.7	3.5	0.0	7.0	250	1749	29787	31087	0.07	2.65	1.03	0.5	3.6	0.4	2.1	0.4	0.2	6.2	0.5
Soytone, Bacto	9.4	3.1	0.33	292.5	12.0	4.6	0.2	7.2	550	1610	22200	34040	0.17	2.33	0.82	0.4	2.5	2.1	2.8	0.3	0.2	5.5	0.4
TC Yeastolate UF	10.6	6.5	0.61	124.2	13.3	2.1	1.0	7.0	247	267	60940	3716	0.52	0.89	2.46	5.5	5.7	1.9	3.2	1.3	2.1	6.4	0.2
TC Yeastolate, Bacto	10.7	6.0	0.56	143.0	11.7	2.2	0.6	7.0	228	250	50850	8190	0.30	0.49	2.63	4.6	4.6	1.7	2.4	1.2	1.8	4.8	0.2
Yeast Extract	11.4	6.9	0.60	67.6	13.1	1.0	0.2	7.0	230	799	58013	1003	0.07	0.65	3.73	5.7	6.2	2.0	3.0	1.0	2.2	5.9	0.2
Yeast Extract, Bacto	10.9	6.0	0.55	163.3	11.2	3.1	0.1	6.7	130	750	31950	4900	0.38	0.09	3.27	4.4	5.6	1.4	2.6	1.0	1.6	5.3	0.2
Yeast Extract, LD	8.1	6.1	0.75	121.3	17.5	0.3	0.1	7.0	254	649	55700	1683	0.12	0.96	2.11	4.7	5.1	1.8	2.6	1.2	1.9	5.2	0.2
Yeast Extract, UF	10.7	6.0	0.56	108.2	18.2	0.7	0.0	7.0	191	558	59240	1244	0.13	1.02	2.70	4.8	5.4	1.5	2.6	1.2	1.7	5.4	0.2

* = Partially destroyed during hydrolysis

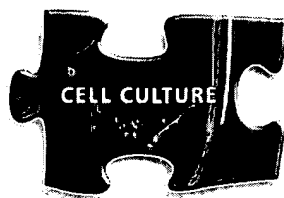
0.0 = Below limits of detection

For test methods see Definition of Methods section on page 61.

Free Amino Acids / Total Amino Acids

	Glutamic Acid (% Free)	Glutamic Acid (% Total)	Glutamine (% Free)	Glycine (% Free)	Glycine (% Total)	Histidine (% Free)	Histidine (% Total)	Isoleucine (% Free)	Isoleucine (% Total)	Leucine (% Free)	Leucine (% Total)	Lysine (% Free)	Lysine (% Total)	Methionine (% Free)*	Methionine (% Total)	Phenylalanine (% Free)	Phenylalanine (% Total)	Proline (% Free)	Proline (% Total)	Serine (% Free)	Serine (% Total)*	Threonine (% Free)	Threonine (% Total)	Tryptophan (% Free)	Tyrosine (% Free)	Tyrosine (% Total)	Valine (% Free)	Valine (% Total)
	2.3	11.7	0.6	0.3	2.7	0.2	1.5	1.0	3.4	2.3	4.6	0.4	4.2	0.6	0.7	1.3	3.0	0.2	3.0	0.9		0.7	1.5	0.2	0.9	2.0	1.0	3.5
	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	0.3	5.9	0.0	0.2	1.5	0.3	0.8	0.2	1.3	0.8	2.3	1.2	2.4	0.2	0.2	0.2	1.4	0.1	1.8	0.2	0.5	0.1	0.5	*	0.2	0.8	0.1	1.5
	0.4	6.5	0.0	0.2	1.8	0.1	0.9	0.2	1.6	0.9	2.7	1.5	2.8	0.2	0.3	0.3	1.6	0.1	1.9	0.3	0.6	0.1	0.6	0.1	0.3	1.0	0.1	1.7
	5.0	6.6	0.5	0.9	1.9	0.7	0.8	1.4	2.3	2.5	3.2	1.7	3.5	0.8	*	1.6	1.8	0.7	1.9	2.2	*	0.9	1.1	0.4	0.5	0.6	1.7	2.7
	3.1	6.8	0.4	0.6	1.7	0.6	0.9	1.5	2.0	2.4	2.8	1.7	2.9	0.6	*	1.7	1.8	0.5	1.3	1.8	*	0.8	1.0	0.4	0.6	0.8	1.6	2.2
	0.7	6.9	0.2	0.1	2.2	0.5	1.3	0.9	2.6	2.2	3.9	2.6	3.4	0.4	*	1.3	2.4	0.2	2.6	0.3	1.2	0.5	1.0	0.1	1.0	0.8	1.0	2.8
	0.4	8.9	0.1	0.2	2.1	0.2	1.1	0.6	2.8	1.7	4.3	1.9	2.9	0.3	0.5	1.2	3.1	0.2	2.0	0.3	1.4	0.2	1.1	0.2	1.3	1.3	0.4	2.7
	7.3	11.0	0.2	1.5	3.0	0.6	1.5	2.1	3.2	3.0	4.0	2.5	5.1	0.8	0.9	2.4	2.9	1.1	2.0	1.7	1.5	1.5	1.7	0.9	0.1	0.9	2.7	4.0
	6.6	8.7	0.3	1.3	2.7	0.5	1.1	2.1	3.6	3.5	4.9	2.3	4.2	0.8	0.8	2.3	3.3	0.9	1.8	1.5	1.4	1.3	1.4	0.6	0.8	0.9	2.4	3.7
	7.3	11.0	0.1	1.6	3.3	0.3	1.4	2.5	4.7	4.0	6.2	2.7	4.9	0.9	1.1	2.7	4.4	1.3	2.3	1.3	1.9	1.7	1.8	0.7	0.9	1.2	3.0	4.8
	6.6	9.4	0.2	1.0	3.0	0.4	1.3	1.8	3.0	3.0	4.1	1.9	4.6	0.6	0.8	2.0	2.6	0.8	2.0	1.3	1.6	1.1	1.6	0.5	0.8	1.2	2.2	3.5
	6.4	9.6	0.35	1.3	2.8	0.5	1.2	2.1	2.8	3.5	3.9	2.4	4.2	0.8	0.8	2.3	2.5	0.9	1.9	1.5	1.5	1.4	1.5	0.6	0.7	0.8	2.5	3.3
	6.8	10.0	0.3	1.3	2.9	0.6	1.2	1.8	3.8	2.8	4.7	2.2	4.6	0.7	0.8	2.1	3.6	0.9	1.9	1.6	1.7	1.3	1.6	0.5	0.5	0.8	2.4	4.1

CHEMICALLY DEFINED MEDIA



Chemically defined media contain known quantities of only chemically defined ingredients added to purified water for the cultivation of microorganisms, mammalian or insect cells. Defined media include no complex ingredients such as proteins, hydrolysates, animal-derived ingredients, or constituents of unknown composition. There are benefits to using chemically defined media that vary depending on the type of cells cultured and the purpose and scope of application, which range from laboratory scale metabolic studies to production scale fermentation or cell culture.

The absence of animal-derived components, desirable from a regulatory standpoint due to concerns over BSE/TSE, is a major advantage to chemically defined media. If a medium contains only chemicals, it contains no prions, the proteinaceous infective particles and causative agents in BSE/TSE. Reproducibility is another advantage to using chemically defined media; all the components of a defined medium have known chemical structures, which allows for consistent performance of cells in the medium.

Chemically defined formulations offer advantages over complex media related to physical properties of proteins and polypeptides. Chemically defined media for biotransformations offer greater simplicity of both downstream processing and the analysis of biotransformation end products.¹ This is also true for cell culture media; using serum-free and protein-free cell culture media can reduce complications in downstream processing. Chemically defined media also feature greater translucency and lower foaming tendency than do complex, protein-containing media which may cause cloudiness and foam.² Complex media ingredients can introduce endotoxins or inhibitors to an industrial fermentation, so chemically defined media are utilized for processes such as enzyme production that are sensitive to inhibitors.³ Vegetable protein hydrolysates and yeast extract for cell culture applications are often ultrafiltered in order to reduce endotoxin levels.

Despite several advantages, chemically defined media are rarely used in industrial fermentations because complex media usually allow higher yields at lower cost.⁴ Complex ingredients that are inexpensive by-products of food and agriculture industries will provide a majority of nutrients needed for bacterial and yeast fermentation. Each microorganism has a specific set of nutritional requirements that may add a long list of expensive growth factors such as L-amino acids and vitamins to a defined formulation.⁵ Chemically defined media must be optimized specifically for each individual organism, and the design time may be quite lengthy and expensive. Even then, after an extended media development period, the optimized defined media may produce lower yields than will a complex medium.

Semi-defined media can provide a balance between maximum performance and minimum downstream processing issues. Semi-defined media are prepared by adding a small amount (from 0.05 to 0.5%) of a complex ingredient, such as a protein hydrolysate or yeast extract, to a chemically defined medium. The small amount of complex material in a semi-defined medium may provide enough nutrients to enhance growth of microorganisms without interfering with recovery or analysis of products.^{1,4} Utilizing ultrafiltered peptones or extracts may reduce difficulties with downstream processing while providing cells with necessary nutrients. Besides reducing endotoxin levels, ultrafiltration contributes to solubility and ease of filtration of a protein product.

Chemically defined media are very well suited to research purposes at laboratory scale. Reproducibility from working with known constituents makes chemically defined media useful for studying cells' metabolic pathways and nutritional requirements for growth and product formation. Chemically defined media can be optimized for yield or performance by individually controlling the ingredients, especially any possible limiting nutrients, in the formulation. Chemically defined media are also valuable as basal media for screening various complex ingredients such as hydrolysates and extracts. Knowledge gained from biochemical studies and peptone analyses can drive further media improvement and scale-up to production.

BD offers several chemically defined media formulated from components chosen based on purity and quality standards. M9 Minimal Salts, 5x, is a minimal chemically defined dehydrated culture medium that—with the addition of dextrose—is optimized for *Escherichia coli*. The Yeast Nitrogen Base products are minimal chemically defined dehydrated culture media used for yeast molecular genetics studies. M9 Minimal Salts and the Yeast Nitrogen Base products are tested to ensure product quality and lot-to-lot consistency using various physical, chemical and growth support tests.

References

1. Michels and Rosazza. 1999. Methods for biocatalysis and biotransformations. In Demain, Davies (ed.), Manual of industrial microbiology and biotechnology, 2nd ed. American Society for Microbiology, Washington, DC.
2. Nagodawithana and Wasileski. 1998. Media design for industrial fermentations. In Nagodawithana and Reed (ed.), Nutritional requirements of commercially important microorganisms, Estekay Associates, Inc., Milwaukee, WI.
3. Pasupuleti and van Schie. 1998. Production of enzymes. In Nagodawithana and Reed (ed.), Nutritional requirements of commercially important microorganisms, Estekay Associates, Inc., Milwaukee, WI.
4. Dahod. 1999. Raw materials selection and medium development for industrial fermentation processes. In Demain, Davies (ed.), Manual of industrial microbiology and biotechnology, 2nd ed. American Society for Microbiology, Washington, DC.
5. Cote. 1999. Media composition, microbial laboratory scale. In Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York.

DIFCO™ M9 MINIMAL SALTS, 5X

Product Description

Difco™ M9 Minimal Salts, 5x is used in preparing M9 Minimal Medium which is used for cultivating recombinant strains of *Escherichia coli*. M9 Minimal Salts, 5x is a minimal chemically defined dehydrated culture media comprised only of ingredients with known chemical structures.

Sodium phosphate and potassium phosphate are present as buffering agents. Ammonium chloride is a source of nitrogen for cellular systems. Sodium chloride provides essential ions. Glucose may be added as a source of carbohydrate. Supplementing the medium with magnesium and calcium increases the growth of recombinants.

Applications

M9 Minimal Salts, 5x is a 5x concentrate that is diluted to a 1x concentration and supplemented with an appropriate carbon and energy source, such as dextrose, to provide a minimal, chemically defined medium that contains only those ingredients essential for the growth of *E. coli*. The medium will support the growth of "wild-type" strains of *E. coli* and various other bacteria. M9 Minimal Medium may be supplemented with magnesium sulfate, calcium chloride, and other nutrients for increased growth of microorganisms.

M9 Minimal Medium is useful for maintaining positive selection pressure on plasmids coding for the ability to produce essential substances such as amino acids or vitamins. M9 Minimal Medium is also used to maintain stocks of F'-containing bacteria for use with M13. The medium can be supplemented with specific amino acids or other metabolites, allowing for selection of specific auxotrophs. Consult appropriate references for recommended test procedures.¹⁻³

M9 Minimal Medium may be used as a chemically defined basal medium for screening peptones as nitrogen sources for the cultivation of various microorganisms.

Formula Per Liter

M9 Minimal Salts, 5x

Disodium Phosphate (anhydrous) ...	33.9 g
Monopotassium Phosphate	15.0 g
Sodium Chloride	2.5 g
Ammonium Chloride	5.0 g
Final pH 6.8 +/- 0.2 at 25°C	

Physical Characteristics

Difco M9 Minimal Salts, 5x is a white, free-flowing, homogeneous powder.

Availability

Difco™ M9 Minimal Salts, 5x 248510, 500g

References

1. Davis, Dignier and Battey. 1986. Basic methods in molecular biology. Elsevier, New York, NY.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
3. Treco and Lundblad. 1997. Preparation of yeast media. In Ausubel (ed.), Short protocols in molecular biology, Wiley, New York, NY.

DIFCO™ YEAST NITROGEN BASE

DIFCO™ YEAST NITROGEN BASE W/O AMINO ACIDS

DIFCO™ YEAST NITROGEN BASE W/O AMINO ACIDS AND AMMONIUM SULFATE

Product Description

The yeast nitrogen bases are minimal chemically defined dehydrated culture media.

Yeast Nitrogen Base (YNB) contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of carbon. YNB is a defined base composed of salts, vitamins, amino acids and trace elements with ammonium sulfate as the sole nitrogen source in this basal medium. Addition of a carbon source is required.

Yeast Nitrogen Base w/o Amino Acids contains all essential vitamins and inorganic salts necessary for the cultivation of yeasts except the amino acids histidine, methionine, tryptophan and a source of carbon. As in YNB, ammonium sulfate is the sole source of nitrogen in this basal medium. Addition of a carbon source is required.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate has the same formulation as the YNB w/o Amino Acids except that ammonium sulfate as a source of nitrogen has been omitted. Addition of a carbon source is required.

Applications

Chemically defined growth media are useful tools for screening yeast strains and selecting for growth requirements.

Yeast Nitrogen Base is used for classifying yeasts based on carbon assimilation.

Yeast Nitrogen Base w/o Amino Acids, which lacks the amino acids histidine, methionine and tryptophan, is used for classifying yeasts based on amino acid and carbohydrate requirements.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate, which lacks amino acids and ammonium sulfate, is used for classifying yeasts based on carbon and nitrogen requirements.

Yeast Nitrogen Base w/o Amino Acids and Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate are prepared according to modifications of Wickerham's Yeast Nitrogen Base formulation.¹⁻³ These media are utilized in many applications for the study of yeasts in molecular genetics. Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate is recommended as a base in preparing several synthetic minimal media and synthetic complete and dropout media for yeast studies.⁴⁻⁶

Physical Characteristics

Yeast Nitrogen Base is off-white, free-flowing, homogeneous.

Yeast Nitrogen Base w/o Amino Acids is off-white, free-flowing, homogeneous.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate is light yellowish beige, free-flowing, homogeneous.

Formula Per Liter

	Yeast Nitrogen Base	Yeast Nitrogen Base w/o Amino Acids	Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate
Nitrogen Sources			
Ammonium Sulfate	5.0 g	5.0 g	--
Amino Acids			
L-Histidine Monohydrochloride	10 mg	--	--
LD-Methionine	20 mg	--	--
LD-Tryptophan	20 mg	--	--
Vitamins			
Biotin	2 µg	2 µg	2 µg
Calcium Pantothenate	400 µg	400 µg	400 µg
Folic Acid	2 µg	2 µg	2 µg
Inositol	2000 µg	2000 µg	2000 µg
Niacin	400 µg	400 µg	400 µg
p-Aminobenzoic Acid	200 µg	200 µg	200 µg
Pyridoxine Hydrochloride	400 µg	400 µg	400 µg
Riboflavin	200 µg	200 µg	200 µg
Thiamin Hydrochloride	400 µg	400 µg	400 µg
Compounds supplying trace elements			
Boric Acid	500 µg	500 µg	500 µg
Copper Sulfate	40 µg	40 µg	40 µg
Potassium Iodide	100 µg	100 µg	100 µg
Ferric Chloride	200 µg	200 µg	200 µg
Manganese Sulfate	400 µg	400 µg	400 µg
Sodium Molybdate	200 µg	200 µg	200 µg
Zinc Sulfate	400 µg	400 µg	400 µg
Salts			
Monopotassium Phosphate	1.0 g	1.0 g	1.0 g
Magnesium Sulfate	0.5 g	0.5 g	0.5 g
Sodium Chloride	0.1 g	0.1 g	0.1 g
Calcium Chloride	0.1 g	0.1 g	0.1 g
Final pH	5.4 +/- 0.2 at 25°C	5.4 +/- 0.2 at 25°C	4.5 +/- 0.2 at 25°C

Availability

Difco™ Yeast Nitrogen Base 239210, 100g

Difco™ Yeast Nitrogen Base w/o Amino Acids 291940, 100g

Difco™ Yeast Nitrogen Base w/o Amino Acids 291920, 2kg

Difco™ Yeast Nitrogen Base w/o Amino Acids 291930, 10kg

Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate 233520, 100g

Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate 233510, 10kg

References

1. Wickerham. 1951. Taxonomy of yeasts. Technical bulletin No. 1029. U.S. Dept Agriculture, Washington, DC.
2. Wickerham. 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. *J. Bacteriol.* 52:293.
3. Wickerham and Burton. 1948. Carbon assimilation tests for the classification of yeasts. *J. Bacteriol.* 56:363.
4. Treco and Lundblad. 1997. Preparation of yeast media. In Ausubel (ed.), *Short protocols in molecular biology*, Wiley, New York, NY.
5. Sherman. 1991. Getting started with yeast. In Guthrie and Fink (ed.), *Methods in enzymology*, vol. 194, guide to yeast genetics and molecular biology, Academic Press, Inc., New York, NY.
6. Warren. 2003. Candida, Cryptococcus and other yeasts of medical importance. In Murray (ed.), *Manual of clinical microbiology*, 8th ed., American Society for Microbiology, Washington, DC.

DEFINITION OF METHODS

Analytical tests used in data gathering for this manual are described below.

The **AN/TN** ratio gives an estimate of the degree of protein hydrolysis.

Ash values were measured after heating at 650°C overnight. Ash values refer to the non-combustible portion of the sample and roughly correspond to the mineral content of the sample.

Total Carbohydrate percentage was calculated by colorimetric assay.

Chloride, Sulfate and Phosphate percentages were determined by ion chromatography.

Elemental analysis was determined by ICP (Inductively Coupled Plasma) using a Thermo Jarrell Ash instrument or equivalent.

Endotoxin values were determined by a quantitative kinetic chromogenic method.

Free Amino Acids are defined as amino acids that are not part of a protein or peptide chain. The amino acids were measured using the Waters AccQ•Tag™ Method. The AccQ•Tag Method is based on the derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimide-activated heterocyclic carbamate.

Labsystems BioScreen C is a 200-well incubating kinetic optical density reader. Media were inoculated with approximately 100 CFU per 200 µL fill in each well. OD readings were averaged from 4 duplicate wells.

L.O.D. (Loss on Drying) test procedure is based on the method described in *The United States Pharmacopeia*.¹ There are some modifications to the procedure.

Molecular weight distribution was determined by size-exclusion chromatography using a silica-based column and a SDS/phosphate buffer mobile phase.

Nucleotide quantitation (hypoxanthine & thymidine) was determined by reverse-phase HPLC using a silica-based column and a phosphate/methanol gradient.

pH was measured in a 1% solution after autoclaving.

Sodium Chloride was determined by silver nitrate/potassium thiocyanate titration method.

Total Amino Acids were measured by the same method as the Free Amino Acids after an acid hydrolysis at 110°C for 20 hours. Asparagine, cystine, glutamine and tryptophan are destroyed during the hydrolysis. The asparagine, cystine, glutamine and tryptophan values are not reported for Total Amino Acids. Methionine and serine are partially destroyed during the hydrolysis.

References:

1. United States Pharmacopeial Convention, Inc. 2004. *The United States pharmacopeia 27/The national formulary 22—2004*. United States Pharmacopeial Convention, Inc., Rockville, Md.

PRODUCT LISTING

Product Name	100 g	454 g	500 g	2 kg	5 lb (2.3 kg)	10 kg	25 lb (11.3 kg)	25 kg	50 kg
Acidicase™ Peptone			211843						
Beef Extract Powder			212303						
Beef Extract, Desiccated, Bacto™			211520						
Biosate™ Peptone		211862					294312		
Brain Heart Infusion, Bacto™	237400		237500	237200		237300			
Brain Heart Infusion, Porcine			256120			256110			
Casamino Acids, Bacto™			223050	223020		223030			
Casamino Acids, Technical, Bacto™			223120			223110			
Casitone, Bacto™			225930			225910			
Difco™ Springer™ DS100 Soy Peptone UF			220515			220516			
Gelysate™ Peptone		211870							
Difco™ M9 Minimal Salts 5X			248510						
Malt Extract, Bacto™			218630			218610			
Neopeptone, Bacto™			211681			211680			
Peptone, Bacto™	211840		211677	211820		211830			
Peptone, Bitek™						254820			
Phytone™ Peptone		211906			298147	292450			
Phytone™ Peptone UF			210931			210936			
Polypeptone™ Peptone		211910				297108			
Proteose Peptone No. 2, Bacto™			212120			212110			
Proteose Peptone No. 3, Bacto™			211693	212220		212230			211692
Proteose Peptone No. 3, Bitek™							253720		
Proteose Peptone No. 4, Bacto™						211715			
Proteose Peptone, Bacto™			211684			212010			
Proteose Peptone, Bitek™						253310			
Select APS™ LB Broth			292438			212484			
Select APS™ Super Broth			212485			212486			
Select Soytone			212488			212489			
Soytone, Bacto™			243620			243610			
TC Lactalbumin Hydrolysate, Bacto			259962			259961			
TC Yeastolate UF			292804			292805			
TC Yeastolate, Bacto™	255772					255771		292731	
Thiotone™ E Peptone			212302						
Trypticase™ Peptone		211921			211922		211923		
Tryptone, Bacto™			211705	211699		211701			
Tryptone, Bitek™						251420			
Tryptose, Bacto™			211713			211709			
Yeast Extract		211929			211930		211931		
Yeast Extract, Bacto™			212750	212720		212730			212710
Yeast Extract, LD			210933			210941			
Yeast Extract, UF			210929			210934			
Yeast Nitrogen Base	239210								
Yeast Nitrogen Base w/o Amino Acids	291940			291920		291930			
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate	233520					233510			



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